THE ANALYST

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

JOINT MEETING

A JOINT Meeting of the Society with the Fine Chemicals Group of the Society of Chemical Industry was held at 6.30 p.m. on Friday, March 18th, 1960, at the School of Pharmacy, Brunswick Square, London, W.C.1. The Chair was taken by the President of the Society for Analytical Chemistry, Mr. R. C. Chirnside, F.R.I.C.

The subject of the meeting was "Techniques of Automatic Analysis" and the following papers were presented and discussed: "Automatic Analysis in the Chemical Industry," by R. M. Pearson, A.R.I.C.; "Automation in Clinical Biochemistry," by I. D. P. Wootton, M.A., M.B., Ph.D., F.R.I.C.

ORDINARY MEETING

AN Ordinary Meeting of the Society was held at 7 p.m. on Wednesday, April 6th, 1960, in the Meeting Room of the Chemical Society, Burlington House, London, W.1. The Chair was taken by Dr. D. C. Garratt, F.R.I.C., Vice-President.

The following papers were presented and discussed: "A Combined Gravimetric and Photometric Procedure for the Determination of Silica in Silicate Rocks and Minerals," by P. G. Jeffery, M.Sc., Ph.D., D.I.C., A.R.C.S., F.R.I.C., and A. D. Wilson, B.Sc.; "The Estimation of Trimethylene Glycol in Glycerol by Gas Chromatography," by J. Clifford, B.Sc., M.A.; "Direct Colorimetric Determination of Trace Chloride," by T. Nash, M.A., B.Sc., A.R.I.C.

DEATHS

WE record with regret the deaths of

Francis William Frederick Arnaud.
Romily David Thomas Ernest Chandler.

SCOTTISH SECTION

AN Ordinary Meeting of the Section was held at 7.15 p.m. on Friday, February 26th, 1960, at the Central Hotel, Glasgow, C.1. The Chair was taken by the Chairman of the Section, Mr. A. N. Harrow, A.H.-W.C., F.R.I.C.

The following papers were presented and discussed: "Analytical Methods in the Hygienic Control of Industrial Atmospheres," by J. C. Gage, B.Sc., Ph.D., F.R.I.C.; "Analytical Problems in the Isolation and Measurement of Traces of Radioactivity in Foods," by Professor J. Hawthorn, B.Sc., Ph.D., A.R.C.S.T., F.R.I.C.

MIDLANDS SECTION

A JOINT Meeting of the Section with the Birmingham and Midlands Section of the Royal Institute of Chemistry was held at 7 p.m. on Wednesday, February 24th, 1960, in the Department of Chemistry, The University, Edgbaston, Birmingham, 15. The Chair was taken by the Chairman of the Midlands Section, Dr. S. H. Jenkins, F.R.I.C., F.Inst.S.P.

The following paper was presented and discussed: "Some Analytical Aspects of Reactions in Certain Non-aqueous Solutions," by Professor V. Gutmann.

AN Ordinary Meeting of the Section was held at 6.30 p.m. on Wednesday, March 9th, 1960, in the Mason Theatre, The University, Edmund Street, Birmingham, 3. The Chair was taken by the Chairman of the Section, Dr. S. H. Jenkins, F.R.I.C., F.Inst.S.P.

The following paper was presented and discussed: "Plant Growth Promoting Substances —Some Analytical Aspects," by Professor R. L. Wain, D.Sc., Ph.D., F.R.I.C.

MICROCHEMISTRY AND BIOLOGICAL METHODS GROUPS

THE Sixteenth Annual General Meeting of the Microchemistry Group was held at 6 p.m. on Friday, February 19th, 1960, at the Postgraduate Medical School, Ducane Road, London, W.12. The Chair was taken by the Chairman of the Group, Mr. F. Holmes, B.Sc., A.R.I.C. The following Officers and Committee Members were elected for the forthcoming year:—*Chairman*—Mr. F. Holmes. *Vice-Chairman*—Mr. C. Whalley. *Hon. Secretary*—Mr. D. W. Wilson, Department of Chemistry, Sir John Cass College, Jewry Street, Aldgate, London, E.C.3. *Hon. Treasurer*—Mr. G. Ingram. *Members of Committee*—Miss M. Corner, Messrs. C. B. Dennis, C. A. Johnson, R. Magee, D. F. Phillips and H. C. J. Saint. Dr. L. H. N. Cooper and Mr. H. Childs were re-appointed as Hon. Auditors.

The Annual General Meeting was followed at 7 p.m. by a Joint Meeting of the Microchemistry Group with the Biological Methods Group. The Chair at this meeting was taken by the Chairman of the Biological Methods Group, Dr. J. I. M. Jones, F.R.I.C. The following papers were presented and discussed: "Micro-analysis in Clinical Biochemistry," by Professor E. J. King, M.A., D.Sc., F.R.I.C.; "Completely Automatic Methods in Microanalysis," by I. D. P. Wootton, M.A., M.B., Ph.D., F.R.I.C.; "Automatic Titration Apparatus," by Ruth Haslam, M.B., D.C.P., and I. D. P. Wootton, M.A., M.B., Ph.D., F.R.I.C.; "Flame Photometric Analysis of Divalent Cations in Biological Materials," by I. MacIntyre, M.B.; "Optical Rotatory Dispersion," by W. Klyne, M.A., D.Sc.; "Spectrofluorimetric Determination of Alkaline Phosphatase in Micro Quantities of Serum," by D. W. Moss, M.A.

Light-scattering Methods for the Chemical Characterisation of Polymers

A Review

By F. W. PEAKER

(*Chemistry Department, The University, Birmingham*)

SUMMARY OF CONTENTS

Introduction
Turbidimetric titration
Experimental procedure
Treatment of results
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Applications and results

THE structures of macromolecules are in general primarily of interest to physical chemists. In this field of study, as indeed in many others, the techniques developed are of interest to the analyst. The chemistry of the high polymers deals with the study of large molecules; many of these occur naturally, *e.g.*, rubber, proteins and carbohydrates, and similar types of material can be synthesised. In such synthetic work the chemist aims to produce a molecule "tailor-made" for any specific purpose.

A high molecular weight is a common characteristic property of all polymeric materials; indeed most of their properties are dictated by this and other closely related factors. In studying these materials, new and old techniques, both qualitative and quantitative, are involved.

These molecules are built up by repetition of a single monomer unit, A; the structure of the molecule may be linear, for example—

$$\text{A–A–A–A–A–A or A–(A)}_n\text{–A}$$

the molecular weight being controlled by the number of repeating units in each linear chain-like molecule. The number of these units is termed the degree of polymerisation, and thus the molecular weight is found by multiplying this quantity by the molecular weight of the monomer unit. The molecule may be branched, as shown below—

$$\begin{array}{c}\text{A–A–A–A–A–A–A–A–A}\\ \quad|\qquad\quad|\\ \text{(A)}_n\qquad\text{(A)}_{n'}\end{array}$$

and both the number of branches and their lengths may vary. Dissimilar monomers may sometimes polymerise simultaneously to produce a copolymer, which may be one of two types: (*a*) a copolymer having alternating structure and (*b*) one having random structure, *e.g.*—

$$\begin{array}{ll}\text{A–B–A–B–A–B–A–B–A} & \text{A–B–B–A–A–A–B–A–B–B–A}\\ \text{(}a\text{)} & \text{(}b\text{)}\end{array}$$

A block copolymer is a further variant in this class; it has long sequences of monomer units, *viz.*—

$$\text{A–A–A–A–A–A–A–A–B–B–B–B–B–B–A–A–A–A–A}$$

and the number of sequences in each molecule is small. In a branched molecule, the monomer units in the side-chains may be chemically different from those in the main chain—

$$\begin{array}{c}\text{A–A–A–A–A–A–A–A–A–A–A–A–A–A–A}\\ \quad|\qquad\qquad\qquad\quad|\\ \text{(B)}_n\qquad\qquad\text{(B)}_{n'}\end{array}$$

this is a graft copolymer. When the repeating units are polyfunctional or when different types of chemical reaction are possible, the structure may be three-dimensional—

Such a molecule is said to be cross-linked. If cross-linking occurs only in a few places the material is usually soluble and fusible, but if cross-linking is excessive, resulting in a small number of molecules present in a large mass of material, the product is insoluble and infusible.

High polymers in which the stereochemical arrangement of the monomer units is controlled have recently been synthesised.[1,2,3] From the point of view of this paper, they also may be investigated by the methods discussed; in solution, their properties do not differ greatly from those of their non-stereospecific counterparts.

From the methods of synthesis and also from a study of physical properties, we know that in any sample of polymeric material many different sizes of molecules are present. Since it is erroneous to think of this class of materials as being of uniform molecular size, when we refer to their molecular size we are referring to an average value. In general, when there are present N_i moles of material M_i, where in principle i can vary from one to infinity, the number-average molecular weight is calculated on the basis of the fraction by number of each species present. The number-average molecular weight, $\bar{M}_n$, is therefore given by the expression—

$$\bar{M}_n = \frac{N_1 M_1}{\sum_i N_i} + \ldots + \frac{N_i M_i}{\sum_i N_i} = \frac{\sum_i N_i M_i}{\sum_i N_i}$$

If the average is assessed on the basis of the fraction by weight of each species present, the result is the weight-average molecular weight, $\bar{M}_w$, which is given by the expression—

$$\bar{M}_w = \frac{N_1 M_1 M_1}{\sum N_i M_i} + \ldots + \frac{N_i M_i M_i}{\sum N_i M_i} = \frac{\sum N_i M_i^2}{\sum N_i M_i}$$

If the material is monodisperse, only one species i is present and the two averages are identical. In practice, i covers a wide range of values and $\bar{M}_w$ is always greater than $\bar{M}_n$. Further, the ratio of $\bar{M}_w$ to $\bar{M}_n$ is a measure of the range of molecular weights present, *i.e.*, the molecular-weight distribution. Both the value of the mean molecular weight and the molecular-weight distribution are of significance in the characterisation of polymeric material.

In solution, molecules of a material having a fixed molecular weight may vary in shape and size. If dissolved in a good solvent, the molecules will be extended and possess a large end-to-end distance. In a poorer solvent, or if non-solvent is added to the good solvent, the viscosity decreases as a result of a decrease in size of the molecules; finally, if sufficient non-solvent is added, the polymer molecules are precipitated as spherical particles. These particles, if prepared in special circumstances, can be viewed as individual molecules with an electron microscope.[4]

Turbidimetric titration

If non-solvent is added to a solution of a high polymer, the solvent and the non-solvent being miscible in all proportions, precipitation of the polymer occurs at a well defined solvent composition. If the initial-precipitation point, γ_s, is defined, as a volume concentration, by the equation—

$$\gamma_s = \frac{V_n}{V_s + V_n} \quad \ldots \quad (1)$$

in which V_n and V_s are the volumes of non-solvent and solvent, respectively, then, for one polymer only, γ_s is dependent on the concentration of the polymer and the highest molecular weight species present. For polymers differing in chemical composition, but nevertheless soluble in a single solvent, γ_s is also dependent on the chemical composition. Precipitation always occurs over a range of solvent - non-solvent compositions and is dependent on the concentration of the solution and the molecular weight of the polymer. All these facts were established in exploratory work by Staudinger[5] and Schulz and Jirgensons[6]; they also form the basis of the extraction and precipitation methods of separating block copolymers.[7]

The use of precipitation as a quantitative method was first suggested by Morey and Tamblyn,[8] who experimentally established the relationship—

$$\gamma_s = k \log c + f(M) \qquad (2)$$

in which k is constant for any polymer - solvent - non-solvent system and $f(M)$ is a function of the molecular weight. Turbidimetric titration involves precipitation of the polymer and investigation of the amount thrown out of solution at any particular concentration of solvent by measuring the light transmission of the suspended polymer. Fig. 1 shows in diagrammatic form the essentials of the instrument required to carry out these measurements. A Spekker absorptiometer can be simply adapted for work of this type. Instruments have also been specially designed for this purpose,[8,9,10] and that designed by Melville and Stead is perhaps more suitable if a large number of measurements has to be made.[10]

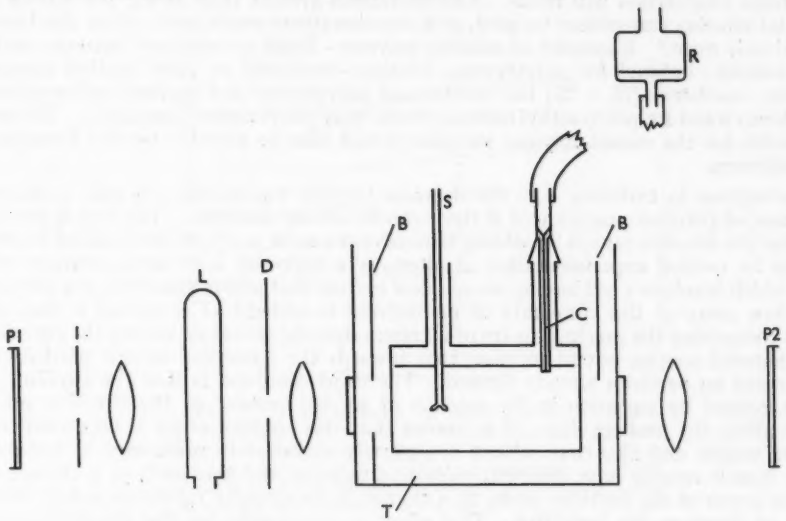

Fig. 1. Modified Spekker absorptiometer used in the turbidimetric titration of high-polymer solutions

Five points, relevant to both instrumentation and experimental procedure, require special consideration.

(*i*) In the earlier work it was usual to add the non-solvent in about 0·5-ml increments, to stir, and to record the transmission measurements until equilibrium was attained; under these conditions it could take many hours to complete a titration. More rapid and reproducible results can be achieved by adding the non-solvent slowly and continuously; for a cell of 50-ml capacity a suitable rate is about 0·002 ml per second.

The addition may be made by using either a capillary or a micro pump.[11,12] It is also argued that continuous addition will more closely correspond to true equilibrium conditions.

(*ii*) All solvents and precipitants should be filtered through a sintered-glass filter (porosity No. 3 or finer) before use.

(*iii*) Provided that the rate of stirring is below a critical maximum it does not appear to influence the size of the precipitated particles; rapid stirring usually promotes coagulation of the polymer, thereby invalidating the results. The tendency towards aggregation varies from system to system, so that no generalisations are possible; however, stirring should be as gentle as is compatible with thorough mixing. The stirrer can be placed in the light beam without causing complications, provided that it does not cause air bubbles to appear in the bulk of the liquid. An air-driven turbine (Quickfit micro stirrer) or variable-speed electric motor will give the required control.

(*iv*) Adequate control of temperature can be achieved by circulating water from a thermostat. If the solvent and non-solvent have an appreciable heat of mixing, this may be troublesome if the non-solvent is not added continuously.

(*v*) The final turbidity and hence the accuracy of the method cannot be increased beyond a certain limit by increasing the concentration of the solution; at high concentrations coagulation will result. Concentrations greater than 10 mg per 100 ml in the initial solution can seldom be used, and concentrations much higher than this have been used only once.[8] Examples of suitable polymer - liquid systems are benzene - methanol or toluene - butanol for polystyrene, benzene - methanol or ethyl methyl ketone and water - methanol (75 + 25) for mixtures of polystyrene and methyl methacrylate and acetone - water for poly(methyl methacrylate) - poly(vinyl acetate) mixtures. The systems suitable for the mixed-polymer samples should also be suitable for the corresponding copolymers.

The increase in turbidity, *i.e.*, the decrease in light transmission, is only a measure of the amount of polymer precipitated if three conditions are satisfied. The first is that a correction for the dilution caused by adding non-solvent can be made on the basis of Beer's law. (This can be verified experimentally; an alternative approach is to use a constant-volume system, which involves a cell having an overflow system that allows mixed solvent and precipitate to flow away at the same rate as non-solvent is added.) The second is that all the particles comprising the precipitate are of identical size and also that, during the intermediate stages, material coming out of solution goes towards the formation of new particles and is not deposited on particles already formed. The third condition is that the swelling of the particles, caused by variation in the amount of solvent present as the titration proceeds, does not affect the particle size. It is known that this swelling effect is dependent on the molecular weight and also that solvent is generally absorbed in preference to non-solvent. The two liquids usually have different refractive indexes, and this leads to a change in the scattering power of the particle, owing to a change in its effective refractive index; this may increase or decrease the turbidity. This effect is responsible for the steady increase or decrease in turbidity when non-solvent is added after precipitation is complete. The effect is, of course, eliminated if a solvent - non-solvent pair having identical refractive indexes can be found.

On the basis of electron micrographs and light-scattering measurements,[13] it seems most probable that the particle size changes during titration. In spite of this results are reproducible; indeed, quantitative results based on the assumption that the above conditions are satisfied give sensible and useful information.

Experimental procedure—

When a modified Spekker instrument is used, the procedure is as described below. The cylindrical cell is filled with an appropriate amount of polymer solution, and an amount of non-solvent, insufficient to cause precipitation, is added. This brings the solution close to the precipitation point. At this stage the liquid in the cell should completely cover the light beam. Precipitant, usually about 10 to 20 ml, is then added continuously, and the photocell outputs are balanced by movement of the drum, this being done at equal intervals

of time. The drum, D (see Fig. 1), is initially set to read an optical density of unity, and the photocells are balanced by adjusting the iris diaphragm, I. If the capillary feed is from a wide-bore container through a large head of liquid, the rate of efflux from the capillary can be assumed to be linear with time. Thus, with drum readings being taken at equal intervals of time, the composition of the liquid mixture in the titration cell is known during the whole precipitation process.

TREATMENT OF RESULTS—

The observed turbidity, $\tau_{obs.}$, is obtained by subtracting the drum reading from unity, and τ_c, the turbidity corrected for dilution, is given by the expression—

$$\tau_c = \frac{\tau_{obs.}(V_s + V_n)}{V_s} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (3)$$

where $\tau_{obs.}$ is related to the intensities of the primary and transmitted beams, I_0 and I, by the relationship—

$$I = I_0 e^{-\tau l} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (4)$$

l being the length of liquid in the cell. For measurements in the same cell, the quantity τl can be used instead of τ. Figs. 2 and 3[14] show the variation in measured turbidity, corrected for dilution, with solvent composition for two polystyrenes differing in molecular weight.

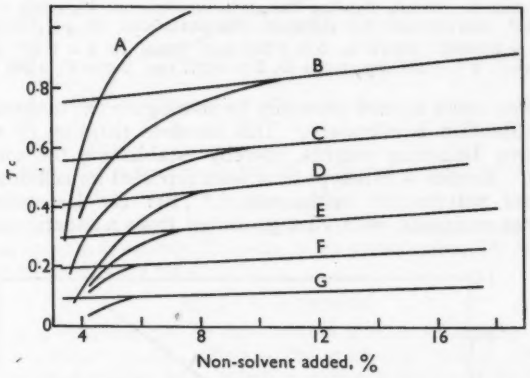

Fig. 2. Graph showing variation in corrected turbidity with added non-solvent for different concentrations of polystyrene (molecular weight = 4×10^6): curve A, $10 \times 0{\cdot}264$ mg; curve B, $7{\cdot}5 \times 0{\cdot}264$ mg; curve C, $5 \times 0{\cdot}264$ mg; curve D, $4 \times 0{\cdot}264$ mg; curve E, $3 \times 0{\cdot}264$ mg; curve F, $2 \times 0{\cdot}264$ mg; curve G, 0·264 mg

The solvent - precipitant mixure was ethyl methyl ketone and water - methanol (75 + 25). It can be seen that the maximum turbidity measured is dependent on the amount of polymer present and also that the turbidity continues to increase after precipitation is complete. This can be attributed to the refractive-index changes in the continuous phase. If the linear portion of such a curve is extrapolated back to zero concentration of non-solvent, the intercept is proportional to the initial concentration of the polymer solution. This is shown in Fig. 4.

The chemical nature of a polymer has a much greater influence on its solubility than has its molecular weight. This can be seen from Fig. 5, in which the percentage change in turbidity is plotted against the percentage of methanol added to benzene solutions of polymers. Curves A and B are for polystyrene and poly(methyl methacrylate), respectively, and curve C is for a mixture of the two polymers in a single solvent. If these two polymers, dissolved in benzene, are simultaneously subjected to ultra-sonic radiation, both are degraded, and some block copolymer is formed by combination of the fragments. The turbidimetric-titration curve for such a system after irradiation is shown in Fig. 6. X is the polystyrene, Y is the

block copolymer and Z is the poly(methyl methacrylate). Any copolymer, random or block, is precipitated over a wide range of solvent compositions, its solubility being primarily determined by chemical composition and only to a secondary degree by molecular weight.

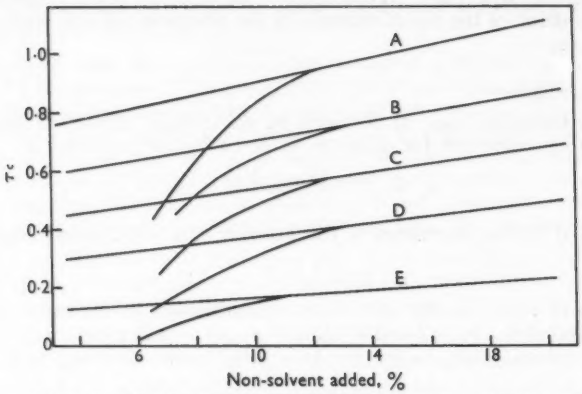

Fig. 3. Graph showing variation in corrected turbidity with added non-solvent for different concentrations of polystyrene ($M_n = 80{,}000$): curve A, $5 \times 0{\cdot}207$ mg; curve B, $4 \times 0{\cdot}207$ mg; curve C, $3 \times 0{\cdot}207$ mg; curve D, $2 \times 0{\cdot}207$ mg; curve E, 0·207 mg

When the titration curve is used primarily to investigate the molecular-weight distribution, preliminary calibration is necessary. This involves titration of several fractionated samples having known molecular weights, thereby establishing the solubility relationship given in equation (3). Results of this type have been reported for cellulose acetate butyrate,[8] cellulose nitrate[15] and poly(methyl methacrylate).[9] For the last-named compound, the distribution curve was compared with that predicted from a kinetic analysis of the polymerisation reaction.

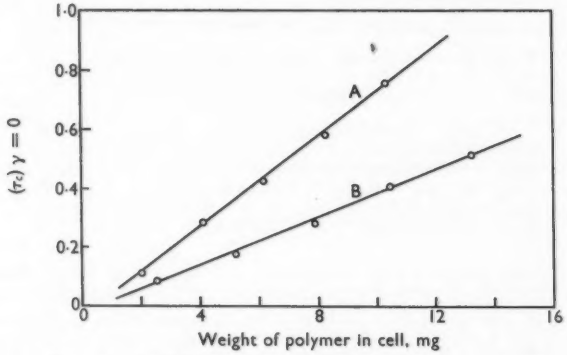

Fig. 4. Graph of $(\tau_c)\gamma = 0$ (*i.e.*, value of corrected turbidity at zero per cent. non-solvent) plotted against the weight of polymer in the titration cell: curve A, molecular weight of polymer = 80,000; curve B, molecular weight of polymer = 4×10^6

There is no doubt that much qualitative information is readily obtainable from measurements of this type. The exact quantitative interpretation is open to question, but it must be remembered that the process of obtaining more exact information, particularly in the field of molecular-weight distributions and the analysis of block copolymers, is time-consuming and not particularly precise[16]; moreover, other methods require much more starting material.

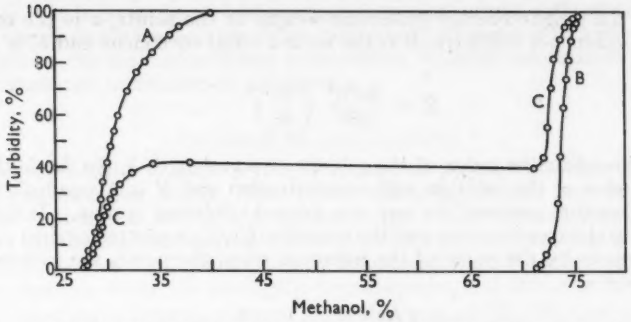

Fig. 5. Variation in turbidity with added non-solvent: curve A, polystyrene; curve B, poly(methyl methacrylate); curve C, a mixture of the two

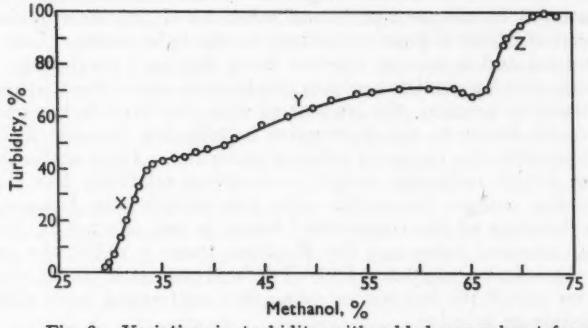

Fig. 6. Variation in turbidity with added non-solvent for a mixture of polystyrene (X), poly(methyl methacrylate) (Z) and a block copolymer of the two (Y)[45]

Light scattering

The turbidity of a medium can be determined from a knowledge of the intensities of the incident and transmitted beams. This is the approach used in turbidimetric titrations when the polymer is precipitated from solution and the resultant turbidity is appreciable, and, because there is a large difference between the two intensities, the turbidity can be measured with fair precision. Solutions of polymers are not visibly turbid, and the alternative approach of measuring the scattered light directly must be used. Photomultiplier cells are generally used for this type of work.

If i_θ is the intensity of the scattered light measured at a distance r from the scattering centre in a direction θ to the light beam, then—

$$\tau = \frac{16\pi\, i_\theta r^2}{3\, I_0\,(1 + \cos^2\theta)} \quad .. \quad .. \quad .. \quad .. \quad (5)$$

The reduced intensity of scattering at an angle θ, R_θ, is defined by the expression—

$$R_\theta = \frac{i_\theta\, r^2}{I_0\,(1 + \cos^2\theta)} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (6)$$

The scattering of light by a solution is a result of the thermal motion of the solute particles, which causes small refractive-index changes in the solution. The molecular weight of the solute is related to the excess scattering of the solution over that of the solvent; the larger the molecular weight of the solute, the greater the optical inhomogeneities produced. This excess of scattering, *i.e.*, the difference between the values of R_θ for the solution and solvent is related to the molecular weight of the solute by the expression—

$$\frac{Kc}{R_\theta} = \frac{1}{\overline{M}_w} + \frac{2Bc}{RT} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (7)$$

in which $\overline{M}_w$ is the weight-average molecular weight of the solute, c is the concentration of the solution in grams per millilitre, B is the second virial coefficient and K is defined by the expression—

$$K = \frac{2\pi^2 n^2}{\lambda^4 N}\left(\frac{\delta n}{\delta c}\right)^2$$

in which n is the refractive index of the solvent at wavelength λ, $(\delta n/\delta c)$ is the variation in the refractive index of the solution with concentration and N is Avogadro's number. The value of K is therefore constant for any one solvent - polymer system. If the scattering is measured at several concentrations and the quantity Kc/R_θ is plotted against c, the molecular weight is then given by the value of the intercept when the points are extrapolated to zero concentration, *i.e.*—

$$\left(\frac{Kc}{R_\theta}\right)_{c=0} = \frac{1}{\overline{M}_w} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (8)$$

It must be stressed that the molecular weight so obtained is the weight-average value.

The light scattered by simple liquids and solutions of polymers having low molecular weights is symmetrical about a position normal to the light beam. This was the type of scattering first studied and is usually referred to as Rayleigh scattering. However, when the diameter of the scattering particle becomes greater than about one-tenth of the wavelength of the light, measured in solution, the amount of light scattered in the backward direction ($\theta > 90°$) is decreased, owing to the destructive interference between light scattered from different parts of the scattering centre (a polymer molecule). Light scattering from solutions of a solute having a high molecular weight is therefore relatively less than the Rayleigh scattering at all finite angles. No matter what the particle size, however, the scattering at 0°, *i.e.*, in the direction of the transmitted beam, is not affected by interference. The ratio between the measured value and the Rayleigh value is called the particle-scattering factor, P_θ. Its value can be calculated for well defined geometric forms, namely, rigid rods, spheres and random coils,[17] the last-named being that conforming most closely to the shape of a polymer molecule in solution.

The exact relationship, applicable in all instances, between the intensity of scattering and the molecular weight is therefore given by the expression—

$$\frac{Kc}{R_\theta} = \frac{1}{\overline{M}_w P_\theta} + \frac{2Bc}{RT} \quad .. \quad .. \quad .. \quad .. \quad (9)$$

P_θ tends towards unity as θ tends to zero and also as $\overline{M}_w$ decreases. Thus P_θ may be unity for all values of θ when $\overline{M}_w$ is sufficiently small, but only when θ tends to zero for large molecular weights.

The angular variation in scattering can be interpreted in terms of the particle-scattering factor or as a dissymmetry factor, z, which is the ratio of the scattering at angles symmetrical about the normal. Thus $z_{45°/135°}$ is the ratio of scattering at 45° to that at 135°. The dissymmetry always has a value greater than or equal to unity at low or zero concentrations. From the experimentally determined values of P_θ or z, by assuming that the particle conforms to one of the geometric forms mentioned earlier, an estimate of the size of the polymer molecule in solution can be made.[17,18,19] A description of the apparatus required and the experimental technique is beyond the scope of this review; for further information the reader is referred to recent books.[12,18] Given a suitable instrument capable of measuring the extremely low light intensities encountered, the main problem is in handling the solutions, which must at all times be free from dust or other contamination that would also cause scattering. Further, the intensity of scattering is several orders of magnitude less than that of the incident beam, and both must be measured with precision to determine the values of R_θ required in the determination of the weight-average molecular weight.

Viscosity measurements on dilute solutions of polymers is the method most widely used for determining molecular weights. Light-scattering studies involve a more exacting experimental technique and are usually more time-consuming. Compared with the osmotic-pressure method, the time-factors are approximately equal, but the apparatus is more complicated. A light-scattering apparatus is, however, less complicated than an ultracentrifuge.

Its chief merit is that it gives the weight-average molecular weight without reference to any other method; the viscosity method has to be calibrated by reference to some other method. At the same time as the molecular-weight information, valuable information about the size of the polymer molecule in solution is obtained.

Applications and results

During the years 1949 to 1955 several fractions of polystyrene were sent to various laboratories throughout the world. This co-operative experiment was initiated by the Macromolecular Commission of the International Union of Pure and Applied Chemistry. Each laboratory carried out independent molecular-weight measurements by several methods; a summary of the recorded results was given by Frank and Mark.[20] Because of the amounts required, these samples could not be highly monodisperse, and this was confirmed by the ratios of weight-average to number-average molecular weights ($\overline{M}_w/\overline{M}_n$) found. For all the samples studied, this ratio was appreciably greater than unity. From this information, one can estimate the error relevant to any one of the more usual methods of molecular-weight determination. By taking results for only one polymer having a molecular weight of average value and by quoting results for only one solvent, namely, ethyl methyl ketone, the mean value of the molecular weight determined osmotically was 235,000, with a spread of ± 15 per cent., $\overline{M}_w$ from light scattering was 330,000 $\pm$ 10 per cent. and the viscometric values showed a variation of ± 10 per cent. When viewed in this way the alternative methods show similar accuracies. At first sight the error may appear to be large, but in view of the nature of the diverse sources of the results, the accuracy should be regarded as satisfactory.

In favourable circumstances it is possible to measure low molecular weights by the light-scattering method. Maron and Lou[21] have confirmed the calibration of their own instrument, based on the use of Ludox, a colloidal dispersion of silica, by measuring the molecular weight of sucrose in aqueous solution. The value arrived at was 338 $\pm$ 6, compared with the known value of 324·2. Work on material having a low molecular weight has also been reported by Rempp and Benoit.[22] The number-average molecular weights of some polyoxy glycols were estimated by end-group analysis and found to be within the range 320 to 10,000. Values based on light-scattering measurements were in close agreement, the ratio $\overline{M}_w/\overline{M}_n$ varying from 1·07 to 1·30 as the molecular weight increased. Again, the closeness of this ratio to the theoretical value of 1·0 for monodisperse material is good evidence for the accuracy of the light-scattering values.

The application of this optical method to the study of high polymers has been dealt with in recently published books.[12,18] Selected references that may be of interest deal with poly(methyl methacrylate),[23] substituted polymethacrylates,[24] poly(vinyl acetate)[25] and polystyrene.[26] In the field of what may be termed naturally occurring high polymers, work has been described on starches,[27] cellulose derivatives,[28] dextrans[29,30] and the fibrin - fibrinogen system.[31] The micelles formed by surface-active agents in aqueous solutions are of a size rendering them suitable for study.[32,33,34]

Polyethylene is an important commercial substance. It is somewhat different from other synthetic polymeric material in that the normal products are soluble only in known solvents at elevated temperatures. This means that modifications to the more usual light-scattering techniques are required.[35,36] Significant results concerning the molecular weights and molecular-weight distributions of these materials have been obtained[35,36]; these results are vital to the fuller understanding of currently competing materials having different melting-points and degrees of crystallinity.

The stability of diesel fuel is a problem of interest to the oil industry. Owing to a tendency towards gum formation, certain oils are subject to troublesome storage problems. The tendency of any one sample towards this undesirable property can be quickly detected by an increase in the turbidity of the oil, which appears during a few days' storage at elevated temperatures.[37] The estimated storage life at ordinary temperature can thus be predicted.

Problems associated with the coagulation of inorganic precipitates, *e.g.*, silver halides,[38] have been studied, and barium sulphate has been the subject of work by Sloan.[39] These systems scatter a great deal of light and do not present many of the experimental difficulties associated with measurements made on solutions. Kerker, Cox and Shoenberg[40] have shown how the particle-size distribution in such systems can be assessed. If the particle size and

density are suitable, this information can be deduced from a study of the variation in scattering measured at a fixed point as the system sediments under gravity.

In a poor solvent, a molecule of a high polymer has a compact structure and there is minimum intermolecular penetration. When a dilute solution of material under these conditions is evaporated, it is likely that the individual molecules will be separated. If the molecular weight is large, then these individual molecules may be seen in an electron microscope; this technique has been successfully used.[41,42,43] An assumption usually made about the spherical particles appearing on the electron micrograph is that they have the same density as that of the bulk material. Hence, after measuring the diameters, the mass of each particle is known, and, after sizing a large number of particles, the average mass can be found. These estimates agree with values determined by other methods. Similarly, for polymers dispersed in an aqueous emulsion, not necessarily as single particles, the particle size can be found by light scattering; the value found agrees with that estimated by electron microscopy.[44]

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The Polarographic Determination of Small Amounts of Tin and Lead in Zirconium and Its Alloys*

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A direct polarographic method is described for the simultaneous determination of from 10 to 200 p.p.m. each of tin and lead in zirconium and Zirconium MV/ATR (an alloy containing 0·5 per cent. each of copper and molybdenum).

The height of the single polarographic wave produced by the combined effect of lead and tin is measured, the diffusion current produced by lead is then suppressed by co-precipitating lead, as sulphate, with barium sulphate, and the resulting wave height produced by tin alone is measured. Interference from copper and molybdenum is prevented by precipitating these elements as ferrocyanides.

The method can readily be applied to the zirconium-containing materials mentioned above; it is simple and rapid and should be applicable to many different metallurgical materials.

THE increased production of zirconium and its alloys for nuclear engineering purposes has necessitated the development of reliable analytical procedures for determining impurities in zirconium-based materials. A typical example is the determination of tin, and an investigation that resulted in the development of a rapid procedure for determining small amounts of both tin and lead in reactor-grade zirconium and its alloys is outlined in this paper.

The United Kingdom Atomic Energy Authority specifications limit the concentration of tin in zirconium and its alloys to a maximum of 200 p.p.m., and a reliable analytical procedure was required to cover the range from, say, 20 to about 200 p.p.m. of tin in these materials. Unfortunately, no entirely satisfactory chemical method was available, although a volumetric procedure[1] had previously been used for this determination; this method, however, was primarily intended for determining alloying amounts of tin and was not satisfactory at concentrations below about 100 p.p.m. of tin. Copper and molybdenum, present in certain alloys, interfere in the determination, and in presence of these alloying metals practical limitations are imposed.

Colorimetric reagents normally used for determining small amounts of tin, particularly in foodstuffs and biological specimens, include toluene-3:4-dithiol,[2] 8-hydroxyquinoline,[3] molybdophosphoric acid and molybdosilicic acid,[4,5] but none of these reagents is entirely satisfactory. They are not specific, and careful preliminary separations are invariably necessary; further, a practical difficulty is associated with converting the tin to the stannous condition, which is necessary before it can be determined.

Turbidimetric methods involving the use of phenylarsonic acid and its derivatives have been recommended for determining small amounts of tin in copper-based materials,[6] but unfortunately these reagents produce a turbidity in presence of even moderate amounts of zirconium.

The well defined polarographic waves produced by stannous ions in *M* hydrochloric acid[7] have been used as a basis for determining tin in a variety of materials, and a procedure embodying these characteristics has recently been applied to the determination of alloying amounts of tin in zirconium alloys[8]; it is essential to reduce the tin and to maintain it in the stannous state before the polarographic determination.

Lingane[9] stated that solutions of stannic ions produce well defined polarographic waves at −0·25 and −0·52 volt against a standard calomel electrode in a hydrochloric acid - ammonium chloride base electrolyte. A direct polarographic procedure incorporating these conditions would obviate the need for reducing the tin before its determination and have the obvious advantages of rapidity and simplicity. The possibility of developing such a procedure for the determination of trace amounts of tin in zirconium and its alloys was therefore investigated.

* Presented at the meeting of the Midlands Section of the Society on Thursday, January 7th, 1960. This paper received the Elwell Award for 1959.

Experimental

Preliminary experiments—

In preliminary tests, solutions containing from 0·02 to 0·1 mg of tin (equivalent to 40 to 200 p.p.m. of tin in a 0·5-g sample) and a blank solution were examined with a cathode-ray polarograph, a base electrolyte containing sulphuric acid, hydrochloric acid and ammonium chloride in a total volume of 25 ml being used. Sulphuric acid was included because it was proposed to dissolve the samples of zirconium in a mixture of sulphuric and hydrofluoric acids, from which hydrofluoric acid would subsequently be removed.

Well defined polarographic waves having a peak at —0·42 volt against a mercury-pool anode were obtained, and wave heights were shown to be proportional to the amounts of tin present. Three typical polarograms recorded with a K1000 cathode-ray polarograph (Southern Instruments Ltd.) are shown in Fig. 1. Further tests showed that the results were unaffected by the presence of 0·5 g of zirconium, and it was confirmed that no tin was lost during solution of the sample, a procedure including treatment with hydrofluoric - sulphuric acid mixture, oxidation by nitric acid and evaporation until fumes of sulphur trioxide were evolved. It was also shown that variation in the amounts of sulphuric acid or hydrochloric acid - ammonium chloride solution added over a wide range (about 2 to 7 ml of each) had no significant effect on the wave height.

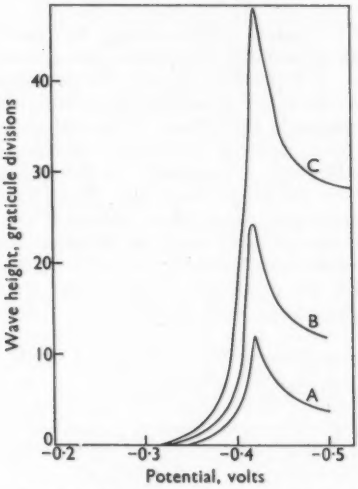

Fig. 1. Typical polarograms for tin: curve A, 0·025 mg of tin (equivalent to 50 p.p.m. in a 0·5-g sample); curve B, 0·05 mg of tin; curve C, 0·10 mg of tin. Sensitivity setting 0·25; volume of solution 25 ml

The fact having been established that a satisfactory relationship between the wave height at —0·42 volt and the amount of tin added could be obtained in presence of zirconium, the effects of likely impurities and common alloying constituents were investigated.

Effect of lead—

Lingane[9] stated that lead ions also produce a well defined polarographic wave at about the same half-wave potential as that of tin (—0·52 volt against a standard calomel electrode) in a hydrochloric acid - ammonium chloride base electrolyte. In view of this, and because up to 50 p.p.m. of lead are usually present in reactor-grade zirconium (the specified limit is 100 p.p.m.), possible interference from lead was investigated.

Solutions containing zirconium and added amounts in the range 50 to 200 p.p.m. each of tin and lead were examined, and it was observed that the wave height at —0·42 volt

increased in proportion to the amount of lead present. The wave height due to lead was about half that produced by an equivalent amount of tin; the height of the single wave produced by tin and lead was therefore equal to the sum of the heights of the waves produced when these metals were examined separately. The tin content of a sample could therefore not be determined without taking into consideration the wave height due to lead.

The problem could have been solved by determining lead separately and making a correction to the height of the single wave produced by tin and lead, but a more direct procedure was preferred.

Baev and Kovalenko[10] found that the diffusion current produced by tin was completely suppressed by the presence of citrate in a hydrochloric acid solution at controlled pH. However, it is difficult to prepare solutions of zirconium in hydrochloric acid alone, and tests showed that, in the base electrolyte used in this work, citrate ions had negligible effect on the diffusion current produced by tin in presence of zirconium.

The established method of isolating small amounts of lead from solution by co-precipitation with barium sulphate[11] was considered as a possible means of overcoming interference from lead. Tests showed that, when a solution of barium chloride was added to the base electrolyte, the diffusion current produced by lead was completely suppressed and the polarographic wave produced by tin was unaffected. Hence, by measuring the height of the polarographic wave before and after co-precipitation of lead, as sulphate, and making a small correction to allow for changes in the volume of the base electrolyte caused by adding barium chloride solution, the simultaneous polarographic determination of tin and lead was possible. The wave produced by up to 500 p.p.m. of lead was completely suppressed by 1 ml of 25 per cent. barium chloride solution, and this volume of reagent was used in subsequent tests.

Solutions containing 0·5 g of zirconium and added amounts of tin and lead were next examined. The height of the single wave produced by tin and lead in a 5-ml portion of the 25 ml of base electrolyte solution was measured, barium chloride solution was added to the remaining 20 ml, and the height of the wave produced by tin alone was measured. After a 5 per cent. positive correction had been applied to the height of the tin wave to compensate for the increase in volume from 20 to 21 ml, lead was determined from the difference between the wave heights; results were satisfactory for the range 25 to 200 p.p.m. each of tin and lead.

EFFECTS OF OTHER IMPURITIES—

Tests to determine the effects of other likely impurities established that tin and lead could be satisfactorily determined in presence of aluminium, cadmium, chromium, cobalt, copper, iron, magnesium, manganese, molybdenum, nickel, titanium, tungsten, vanadium or zinc in amounts well above the specified limits for these impurities in commercial and reactor-grade materials.

One of the principal zirconium alloys in current production, Zirconium MV/ATR, contains about 0·5 per cent. each of copper and molybdenum; the effects of these metals were therefore investigated further.

In acid medium, the half-wave potentials of lead and tin are more negative than those of copper or molybdenum; it was expected, therefore, that the presence of large amounts of these metals would cause interference.

Under the conditions proposed for determining tin and lead, copper produced a polarographic wave at about —0·25 volt, and the presence of more than about 200 p.p.m. of this metal caused low results. Attempts to overcome the effect of copper by forming a complex with tartaric acid or by precipitation with potassium thiocyanate or diethylammonium diethyldithiocarbamate were unsuccessful. When copper was precipitated by potassium ferrocyanide, the diffusion current produced by the copper was completely suppressed, and results for tin and lead were identical with those found in the absence of copper. In the presence of zirconium, 1 ml of 2 per cent. potassium ferrocyanide solution added to the base electrolyte before dilution to 25 ml was sufficient to remove interference from up to about 1 per cent. of copper without any effect on the wave produced by tin and lead (each present in the range 25 to 200 p.p.m.).

The presence of more than about 500 p.p.m. of molybdenum caused the wave produced by tin and lead to be distorted and prevented accurate measurement of its height, but interference from up to about 1 per cent. of this metal could be overcome by forming a molybdenum - tartaro complex or precipitating molybdenum by ferrocyanide. It was further

established that 2 ml of 2 per cent. potassium ferrocyanide solution were sufficient to overcome the combined effects of about 1 per cent. each of copper and molybdenum, and because this single reagent suppressed interference from both metals, it was incorporated in a procedure for application to Zirconium MV/ATR alloy.

Method

Procedure—

Transfer 0·5 g of sample to a platinum dish, and dissolve in 10 ml of diluted sulphuric acid (1 + 1) and about 1 ml of hydrofluoric acid, added dropwise. Oxidise with a slight excess of concentrated nitric acid, evaporate until fumes of sulphur trioxide are evolved, and cool. Carefully wash down the walls of the dish, again evaporate until fumes are evolved, cool, and add about 10 ml of water. Transfer the solution to a 25-ml calibrated flask, and add 5 ml of a mixture of *M* hydrochloric acid and 4 *M* ammonium chloride. If the sample contains more than about 200 p.p.m. of copper or 500 p.p.m. of molybdenum, or both (*e.g.*, Zirconium MV/ATR alloy), add 2 ml of 2 per cent. potassium ferrocyanide solution at this stage. Dilute to the mark. Transfer a 5-ml aliquot to the cell of a cathode-ray polarograph, bubble nitrogen or argon through the solution for about 5 minutes to remove dissolved oxygen, and record a polarogram (start potential −0·2 volt). Measure the height of the wave produced by tin and lead at −0·42 volt.

To the remaining 20 ml of base electrolyte solution add 1 ml of 25 per cent. barium chloride solution. Shake vigorously for about 2 minutes, and examine a portion of this solution polarographically as described above. Measure the height of the wave produced by tin alone at −0·42 volt, make a positive correction of 5 per cent., and deduct the resulting wave height from that of the wave produced by tin and lead.

With each batch of samples, examine a reagent blank solution and a control solution containing the equivalent of 50 p.p.m. of tin and lead. After making an allowance for the reagent blank value, calculate the tin and lead contents of the sample by reference to the wave heights produced by the control solution.

Discussion of results

The proposed method was applied to samples of reactor-grade zirconium and Zirconium MV/ATR alloy, to which the equivalent of 25 and 100 p.p.m. each of tin and lead had been added; the results in Table I show that all recoveries were satisfactory. Replicate determinations were made on typical samples of these materials, and results were reproducible.

Table I

Recovery of tin and lead from zirconium and its alloys

Sample No.	Tin added, p.p.m.	Tin found by proposed method, p.p.m.	Lead added, p.p.m.	Lead found— by proposed method, p.p.m.	Lead found— by alternative method,[12] p.p.m.
Reactor-grade zirconium—					
1	Nil	10, 12, 10	Nil	38, 35, 38	36
	25	35	25	65	—
	25	38	100	140	—
	100	112	25	65	—
	100	115	100	135	—
2	Nil	10, 11, 10, 12, 10, 12	Nil	14, 15, 12, 13, 15, 14	15
Commercial-grade zirconium—					
3	Nil	33, 38, 35	Nil	78, 85, 83	78
Zirconium MV/ATR alloy (0·5 *per cent. each of copper and molybdenum*)—					
4	Nil	<5, <5	Nil	15, 16, 15	18
	25	25	25	38	—
	25	28	100	115	—
	100	105	25	40	—
	100	100	100	118	—
5*	Nil	46, 44, 48, 50, 46, 48	Nil	32, 36, 32, 38, 32, 34	30
6	Nil	5, 7	Nil	25, 28	28

* The standard deviations for the amounts of tin and lead found in this sample by the proposed method were 2·0 and 2·5 p.p.m., respectively.

Lead contents were also determined by an independent method[12] involving conversion to lead diethyldithiocarbamate, extraction of this compound into chloroform from an ammoniacal citrate - cyanide solution, recovery of lead by evaporation to dryness. oxidation of organic matter and polarographic determination of the metal in a nitric acid solution. (This procedure takes about 2 hours to complete after the sample has dissolved.) The results by both methods are compared in Table I, and agreement with expected values is good. No independent method is available for determining trace amounts of tin in zirconium, and it is therefore not possible to give comparative results for this element.

The standard deviation of the proposed method is about 2 p.p.m. for both tin and lead at the 40 p.p.m. level. The method is simple, and tin and lead can be determined in about 30 minutes after solution of the sample. Although primarily developed for the examination of zirconium and its alloys, the method could doubtless be applied, with advantage, to a wide variety of metallurgical and other materials.

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The Determination of Zinc in Metallurgical Materials by Atomic-absorption Spectrophotometry

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The development of a method for determining zinc in metallurgical materials by atomic-absorption spectrophotometry is described. Inter-element effects should theoretically be small, and this has been confirmed, but halogen acids exhibit molecular absorption and should be absent.

The method has been applied to a wide range of typical metallurgical samples, and results compare favourably with those obtained by more time-consuming conventional procedures.

Readily available equipment is used, and the method is rapid and simple to apply.

Several papers[1,2,3] describing the application of atomic-absorption spectrophotometry to the determination of various elements in solution have been published. The main advantages claimed in each of these papers are the simplicity of the method and the lack of interference from other elements.

Walsh[4] has shown theoretically that inter-element effects caused by changes in the temperature of the flame should virtually be overcome when absorption measurements are used. The added advantage that some elements not normally excited in a flame can be determined by absorption measurements makes the method attractive, particularly when the alternative is a time-consuming chemical procedure.

The determination of zinc in metallurgical materials almost invariably necessitates complete removal of the major constituent as a first step and is consequently time-consuming. The potentialities of atomic-absorption spectrophotometry were therefore investigated with the object of providing an improved procedure for determining zinc, particularly in concentrations less than 1 per cent.

Description of apparatus

The equipment used was the prototype of that described by Lockyer and Hames[3] and consisted of a hollow-cathode lamp, with the necessary power supplies, a compressed-air atomiser feeding into a long Meker burner and a Uvispek spectrophotometer; an RCA 1P28 photomultiplier tube was used for detection. Standard equipment supplied stabilised extra-high tension to the photomultiplier tube, and a valve voltmeter bridge circuit similar to that described by Naish and Ramsden[5] was used to measure the output current. With such a circuit the instantaneous output current can be either measured directly or integrated over a period of time by charging a high-quality capacitor and finally measuring the integrated potential.

Experimental

The method used for determining absorption was to measure the intensity of light passing through the flame when distilled water was sprayed and then to measure the reduced intensity when the sample solution was sprayed. If these measurements were made several times, corrections could be effected for any slight drift in the intensity of the lamp, by taking the mean of the intensity values before and after spraying the sample solution into the flame, and either percentage transmission or optical density could be determined. Optical-density measurements were preferred, as a calibration graph prepared from them was almost linear.

The only usable zinc line that exhibits absorption is the resonance line at 2138 A. When the instantaneous-measuring system was used there were short-term fluctuations in the output intensity of this line from the 70/30 brass hollow-cathode lamp. Consequently, all further measurements of intensity were made by integration over 30 seconds.

Preliminary tests showed that absorption was adequate when solutions containing only 10 p.p.m. of zinc (10 μg of zinc per ml of solution) were sprayed into the flame, but it was noted that, after about 10 minutes, there was a considerable reduction in the absorption. This effect was traced to condensation and accumulation of the fine atomised droplets on the walls of the polythene tube connecting the atomiser to the burner. The large drops so formed reduced the effective cross-section of the tube and thereby reduced the amount of atomised solution reaching the burner; when the polythene tube was replaced by a minimum length of glass tube having a wider bore, the effect was overcome.

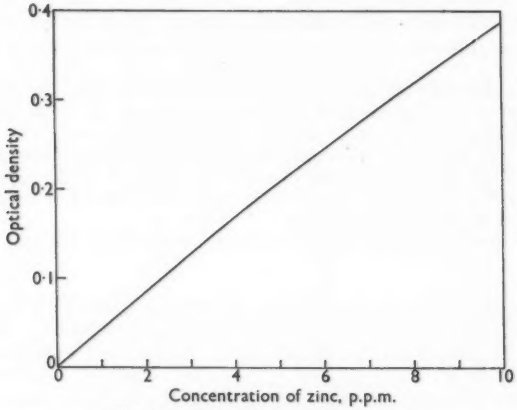

Fig. 1. Calibration graph for zinc

Calibration graphs relating absorption to concentration of zinc were plotted from the results obtained by using standard solutions prepared as described under "Reagents"; these solutions contained 2·5, 5·0, 7·5 and 10·0 p.p.m. of zinc. Ten measurements were made on each solution, and the calibration graph was drawn through the mean points; the general form of the graph is shown in Fig. 1. Slight variations in the slope of the curve occurred from day to day, and, in order to overcome this difficulty, the measured absorptions were corrected proportionally by a factor, so that the standard solution containing 7·5 p.p.m. of

zinc gave a constant corrected absorption. The mean of four such corrected calibration graphs was used as the master graph. In all subsequent determinations of zinc, a standard solution containing 7·5 p.p.m. of zinc was used, and the measured absorptions were corrected proportionately. Variations in absorption were attributed to slight fluctuations in the rate of atomisation, caused by changes in the compressed-air pressure, or to partial blocking of the atomiser jets. There may have been other contributory factors, but full compensation for the effects was achieved by using a standard solution.

With an integration period of 30 seconds, the reproducibility of individual determinations, expressed as a coefficient of variation, was 3 per cent. Because the duration of a single test was short, replicate tests could easily be made, and the mean result of five or ten measurements was usually reported.

INVESTIGATION OF INTERFERENCE—

To determine the extent of any interference caused by the influence of other atoms on the absorption due to zinc, tests were made on (*a*) different concentrations of acid solutions normally used for dissolving metallic samples, (*b*) similar acid solutions containing a known amount of added zinc and (*c*) solutions containing other metals and known amounts of zinc.

Effect of anions and acid concentration—Separate solutions containing known concentrations of hydrochloric, nitric and sulphuric acids and solutions containing mixtures of these acids were prepared. These solutions were examined in the absence and presence of a known amount of added zinc; the results are summarised in Tables I and II.

TABLE I

EFFECT OF ACID CONCENTRATION IN ABSENCE OF ZINC

The optical density of a solution containing 7·5 p.p.m. of zinc was 0·30

Acid	Concentration	Optical density
Nitric	1 + 99	Nil
	1 + 19	
	1 + 9	
	1 + 3	
Sulphuric	1 + 3	0·028
Hydrochloric	1 + 99 (0·1 *N*)	0·025
	1 + 19 (0·5 *N*)	0·075
	1 + 9 (1·0 *N*)	0·21
	1 + 3 (2·5 *N*)	0·52
	0·625 *N**	0·005

* Neutralised with sodium hydroxide before measurements were made.

TABLE II

EFFECT OF ACID CONCENTRATION ON DETERMINATION OF 5 p.p.m. OF ZINC

Concentration of acid	Zinc found in presence of— nitric acid, p.p.m.	sulphuric acid, p.p.m.
1 + 49	4·95	4·60
1 + 19	4·85	4·30
1 + 9	4·65	3·77
1 + 3	4·30	2·90

These results show that concentrations up to 25 per cent. v/v of nitric acid produce no measurable absorption and that sulphuric acid produces only slight absorption at 2138 A. Considerable absorption at 2138 A was produced by solutions of hydrochloric acid, and hydrobromic acid caused a similar effect. Mixtures of acids had a cumulative effect; for example, a mixture of nitric and sulphuric acids, each at a concentration of 10 per cent. v/v, produced only slight absorption, probably due entirely to the sulphuric acid, and results in presence of a mixture of nitric and hydrochloric acids were similar to those in presence of hydrochloric acid alone. This indicates that no effect is produced by interaction of the two acids and that the absorption is entirely due to the concentration of the acid known to cause it. The absorption produced by the halogen acids was considered more fully. From measurements made when unidentified hollow-cathode emission lines (probably argon lines) were used,

it was established that an absorption band between 2100 and 2200 A was produced in presence of hydrochloric, hydrobromic and hydriodic acids. Dilute solutions of hydrofluoric acid also gave indication of similar results. These absorption bands are probably caused by molecular absorption, but are not mentioned in standard books on absorption spectra.[6] Neutralisation of the hydrochloric acid by sodium hydroxide to the change-point of methyl red resulted in almost complete removal of the absorption (see Table I). It appeared, therefore, that the most satisfactory procedure for determining zinc must be based on the absence of halogen acids.

Solutions containing 5 p.p.m. of zinc in various concentrations of nitric and sulphuric acids were prepared, and zinc was determined by comparison with a standard aqueous solution of zinc nitrate. The results (see Table II) indicated that concentrations up to 5 per cent. v/v of nitric acid have negligible effect on the determination. At concentrations of nitric acid between 5 and 25 per cent. v/v, however, there was a progressive decrease in the apparent zinc content. Sulphuric acid, even at a concentration of 2 per cent. v/v, caused a slight reduction in the amount of zinc determined; at a concentration of 5 per cent. v/v the effect was considerable, and it increased with further increases in acid concentration.

Effects of other elements—Solutions containing 5 p.p.m. of zinc and 1000 p.p.m. (0·10 g per 100 ml) of another element were prepared, as far as possible, in the minimum concentration of nitric acid; sulphate was occasionally present and also, when unavoidable, a small amount of chloride. All solutions were compared with a standard solution containing only zinc, as nitrate. No apparent interference was observed from lithium, sodium, potassium, calcium, barium, strontium, copper, cadmium, lead, chromium, manganese, iron, silver, titanium, zirconium, phosphorus, antimony, boron, aluminium, bismuth, cobalt, nickel, arsenic, thorium or tin in the amount stated. A slightly low result (4·75 p.p.m. of zinc) was obtained in presence of magnesium, and silicon produced a significantly low result (2·85 p.p.m. of zinc). In subsequent determinations, silicon was removed as described under "Solution of Sample."

The effects of copper and aluminium, which are major constituents of certain important alloys, were examined more closely, a much greater ratio of metal to zinc being used; the results are shown in Table III. In presence of up to 0·5 g of copper per 100 ml of solution when the ratio of copper to zinc was 1000 to 1 there was no significant reduction in the amount of zinc determined. The presence of 5·0 g of copper per 100 ml of solution and a copper to zinc ratio of 10,000 to 1 produced slightly low results.

TABLE III

EFFECTS OF COPPER AND ALUMINIUM ON DETERMINATION OF 5 p.p.m. OF ZINC

Amount of copper or aluminium present, g per 100 ml	Zinc found in presence of—	
	copper, p.p.m.	aluminium, p.p.m.
0·05	4·98, 5·05	5·10
0·20	4·90	5·08
0·25	4·93	—
0·50	4·82, 4·85	4·95
1·00	4·75	4·48
5·00	4·30, 4·47	—

The presence of up to 0·5 g of aluminium per 100 ml of solution had no significant effect on the accuracy of the determination of 5 p.p.m. of zinc (an aluminium to zinc ratio of 1000 to 1), and the presence of 1·0 g of aluminium (an aluminium to zinc ratio of 2000 to 1) produced a slight decrease in the amount of zinc determined.

A series of mixtures prepared from solutions of pure copper and zinc was examined, and the amounts of zinc determined were in good agreement with those added; the results, the amounts of zinc added and determined being expressed as a percentage of the copper content, were—

Copper present, g	1·0					0·5	0·25	0·10	0·05
Zinc added, % ..	Nil	0·001	0·005	0·010	0·050	0·10	0·20	0·50	1·00
Zinc determined, %	0·0016	0·003	0·008	0·010	0·049	0·097	0·19(5)	0·49	0·98

In some zirconium alloys used for atomic-energy purposes, a maximum zinc content of 0·01 per cent. is specified. Accordingly, further attention was given to the effect of a large amount of zirconium. Two solutions were prepared, each containing 1·0 g of zirconium (purified by the iodide process) and 5 ml each of concentrated nitric and sulphuric acids per 100 ml. To one solution was added 0·1 mg of zinc, equivalent to 0·01 per cent. of zinc in a 1-g sample of zirconium. The zinc contents of the two solutions, based on a 1-g sample, were found to be 0·0027 and 0·0118 per cent., respectively, *i.e.*, the recovery of added zinc was 91 per cent. Interference from zirconium at a ratio of 10,000 to 1 was therefore not excessive, and the accuracy of the determination of zinc at such low levels was regarded as satisfactory. As well as the usual standard aqueous solution of zinc nitrate, a solution containing 0·1 mg of zinc per 100 ml of a mixture of sulphuric and nitric acids, both dilute (1 + 19), *i.e.*, equivalent to 0·01 per cent. of zinc, was used for this determination.

Method

Reagents—

Standard zinc solution—Dissolve 1·000 g of high-purity zinc in 100 ml of dilute nitric acid (1 + 1), cool, and dilute to 1 litre (this solution contains 1·0 mg of zinc per ml). Dilute 100 ml of this solution to 1 litre (this solution contains 0·1 mg of zinc per ml). Separately dilute 25·0-, 50·0-, 75·0- and 100-ml portions of this solution to 1 litre (these solutions contain, respectively, 2·5, 5·0, 7·5 and 10·0 μg of zinc per ml, *i.e.*, 2·5, 5·0 ,7·5 and 10 p.p.m. of zinc).

Secondary standard zinc solution—Place 10·0 ml of the standard solution containing 0·1 mg of zinc per ml in a 1-litre calibrated flask, and dilute to about 500 ml with water. Add 50 ml each of nitric acid, sp.gr. 1·42, and sulphuric acid, sp.gr. 1·84, mix, and allow to cool. Dilute to the mark, and mix. This solution contains 1 p.p.m. of zinc in a mixture of nitric and sulphuric acids, both dilute (1 + 19).

Hydrobromic acid - bromine mixture (9 + 1)—Carefully add 1 volume of bromine to 9 volumes of hydrobromic acid, sp.gr. 1·46 to 1·49, and mix thoroughly.

Acid solvent mixture—Separately add 75 ml each of nitric acid, sp.gr. 1·42, and hydrochloric acid, sp.gr. 1·18, and 150 ml of sulphuric acid, sp.gr. 1·84, to 700 ml of water. Mix thoroughly after each addition, and then allow to cool to room temperature.

Preparation of mean standard graph—

Prepare four standard graphs by using a Hilger atomic-absorption attachment to a Uvispek spectrophotometer with photomultiplier recording. When a copper - zinc lamp is used, the photomultiplier-output current is determined by integration on a capacitor and the resulting potential is measured with a bridge valve voltmeter, satisfactory instrument settings are—

Lamp current—40 mA.
Air pressure to atomiser—15 lb per sq. inch.
Wavelength—2138 A.
Integrating capacitor—0·2 μF.
Integration period—30 seconds.
Photomultiplier voltage—1000 volts.
Slit width—0·18 mm.

Switch on the hollow-cathode lamp, light the burner, and switch on the constant-voltage supply to the photomultiplier tube. Allow 1 hour to elapse in order to attain maximum intensity and stability of emission from the hollow-cathode lamp.

Spray distilled water through the atomiser, integrate the photomultiplier-output current for 30 seconds, and measure the resulting potential (P_o) on the capacitor by using the valve voltmeter. Spray the standard solutions containing 2·5, 5·0, 7·5 and 10·0 p.p.m. of zinc in turn through the atomiser for 30 seconds, and measure each potential (P_t) as before. Repeat this series of tests ten times.

Calculate the optical density for each standard solution from the expression—

$$\text{Optical density} = \log\left(\frac{P_o}{P_t}\right),$$

and construct graphs relating optical density to zinc content. Scale each curve to a standard optical density for 7·5 p.p.m. of zinc (with the equipment described and the conditions listed above, a standard optical density of 0·3 is suitable). From each of the four curves obtained, record the optical-density values corresponding to 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 p.p.m. of zinc. Average the four values so obtained for each concentration, and draw a mean standard graph from these average values.

RECOMMENDED WEIGHT OF SAMPLE—

A solution containing between 1 and 8 p.p.m. of zinc is suitable for atomic-absorption measurements. Use a weight of sample dependent on the zinc content of the alloy, as indicated below.

Zinc content, % ..	0·01 to 0·08	0·04 to 0·16	0·10 to 0·40	0·20 to 0·80	0·40 to 1·60
Weight of sample, g ..	1·0	0·5	0·2	0·1	0·05

For alloys having higher zinc contents, decrease the weight of sample proportionally, and for zinc contents below 0·01 per cent. use an increased weight of sample, *e.g.*, 5·0 g. In the latter instance, however, low results may be obtained owing to the increased viscosity of the solution over that of the standard solution, but this effect can be circumvented by adding to the standard solution an amount of copper, aluminium or other metal equivalent to the weight of the metal in the sample.

SOLUTION OF SAMPLE—

Copper-based alloys containing no tin or silicon—Dissolve the recommended weight of sample in 10 ml of dilute nitric acid (1 + 1), boil for 1 minute to remove nitrous fumes, cool, and dilute to 100 ml.

Copper-based alloys containing tin—Dissolve the recommended weight of sample, *e.g.*, bronze, in 10 ml of hydrobromic acid - bromine mixture (9 + 1), and carefully evaporate to dryness. Add 5 ml of hydrobromic acid - bromine mixture, and again evaporate to dryness. Add 5 ml of nitric acid, sp.gr. 1·42, dropwise, warm to decompose bromides, and again evaporate to dryness. (Note that hydrobromic acid exhibits a definite absorption band over the wavelength used for making atomic-absorption measurements for zinc; it is therefore important to remove all traces of this acid.) Dissolve the residue in 10 ml of dilute nitric acid (1 + 1), and dilute to 100 ml.

Copper-based alloys containing silicon—Transfer the recommended weight of sample to a platinum dish, and dissolve in 10 ml of dilute nitric acid (1 + 1). Add 10 ml of 40 per cent. hydrofluoric acid and 2 ml of sulphuric acid, and evaporate until fumes of sulphur trioxide are evolved to remove all hydrofluoric acid. Cool, dilute with water, transfer to a beaker, and warm until solution is complete (remove any lead sulphate present by filtration). Transfer the solution to a 100-ml calibrated flask, and dilute to the mark with water.

Aluminium-based alloys containing less than 0·5 per cent. of silicon—For aluminium alloys, it is advisable to restrict the weight of sample to 0·5 g or less (see also "Recommended Weight of Sample"). Dissolve the sample in the acid solvent mixture (25 ml of mixture for samples weighing 0·1 to 1·0 g; 10 ml for samples weighing less than 0·1 g). Evaporate the solution until fumes of sulphur trioxide are evolved, cool, and dissolve the residue in 10 ml of dilute nitric acid (1 + 1). Boil for 1 to 2 minutes, cool, and dilute to 100 ml.

Aluminium-based alloys containing more than 0·5 per cent. of silicon—Transfer the recommended weight of sample to a platinum dish, add 10 ml of water, and then cautiously add 40 per cent. hydrofluoric acid dropwise until the sample has dissolved. Add a further 5 ml of hydrofluoric acid, a few drops of nitric acid, sp.gr. 1·42, to dissolve copper, etc., and then 2 ml of sulphuric acid, sp.gr. 1·84. Evaporate until fumes of sulphur trioxide are evolved to remove all hydrofluoric acid. Cool, dilute with water, transfer to a beaker, and add 10 ml of dilute nitric acid (1 + 1). Heat until solution is complete, cool, and dilute to 100 ml.

Zirconium and zirconium alloys—The specified limit for zinc in these materials is usually 100 p.p.m. Transfer 1·0 g of sample to a platinum dish, add about 5 ml of water and then 40 per cent. hydrofluoric acid dropwise until the sample has dissolved. Add a few drops of nitric acid, sp.gr. 1·42, to dissolve any copper or molybdenum and to oxidise carbon, and then add 5 ml of sulphuric acid, sp.gr. 1·84. Evaporate under a radiant heater until fumes of

sulphur trioxide are evolved, cool, dilute with water, and add 5 ml of nitric acid, sp.gr. 1·42. Transfer to a 150-ml beaker, warm until solution is complete, cool, and dilute to 100 ml. Note that, when atomic-absorption measurements for zinc are made on such a solution, the secondary standard solution containing 1 p.p.m. of zinc in dilute sulphuric acid (1 + 19) and dilute nitric acid (1 + 19) should be used as a further check. The zinc content of this solution is determined by reference to the standard solution containing 7·5 p.p.m. of zinc; if there is any appreciable difference from 1 p.p.m., an appropriate correction must be applied.

Miscellaneous alloys and samples—If the recommended weight of sample is used, any material can be analysed for zinc by preparing a solution diluted to 100 ml. It is permissible to use any mineral acid as solvent, or preliminary fusion and then solution of the cooled melt in acid. Silicon, if present, must be removed by treatment with hydrofluoric and sulphuric acids, as described above. However, if a halogen acid is used, care must be taken to ensure that it is completely removed before subsequent dilution of the sample solution. The samples should be in either nitric or sulphuric acid solution or a mixture containing any proportions of these acids. However, if the concentration of sulphuric or nitric acid required to retain the sample in solution is greater than that corresponding to the dilution (1 + 19), changes in viscosity will introduce errors if comparisons are made against aqueous solutions of zinc. An appropriate standard solution prepared in the same concentration of acid as that present in the sample solution should therefore be used for comparison, as described for sample solutions prepared from zirconium and zirconium alloys.

EXAMINATION OF SAMPLE SOLUTION—

Determine the optical density of the sample solution; use the procedure and instrument settings described under "Preparation of Mean Standard Graph." Determine the optical density of the standard solution containing 7·5 p.p.m. of zinc for comparison with each batch of sample solutions. Scale the calculated optical density of the sample solution to correspond to an optical density of 0·30 for the standard solution. Read the amount of zinc in the solution by reference to the mean standard graph, and calculate the percentage of zinc in the sample.

DISCUSSION OF THE METHOD

Interference from acids and metallic salts can be summarised by stating that, apart from the intense absorption band between 2100 and 2200 A produced by halogen acids, the only serious interference is from silicon. If the concentration of acid or metallic salt becomes high enough to cause a decrease in the apparent amount of zinc present, a correction for this can easily be made by using a standard solution containing the same concentration of acid or metallic salt.

The low recovery of zinc from solutions containing an increased concentration of acid or metallic salt was caused by a decrease in the volume of solution passed through the atomiser (owing to changes in density, viscosity, surface tension, etc.); this was confirmed by measuring the amount of liquid delivered to the burner.

The proposed method was applied to the determination of zinc in several copper and aluminium alloys. Zinc was also determined by standard gravimetric, volumetric or polarographic procedures, and a comparison of the results showed good agreement (see Table IV).

Zinc was also determined by the proposed procedure in many samples of zirconium alloys, and, although it was not practicable to carry out the determination by an alternative procedure, the results were consistent and appeared to be reliable. The method has been particularly useful in the examination of zirconium alloys because alternative procedures are lengthy and involve difficult and tedious chemical separations. Although the proposed method is applicable over a wide range of zinc contents in metallurgical materials, it is especially valuable when the zinc content is extremely low; for such samples, accepted chemical methods are difficult to apply and not always entirely satisfactory.

A further application that has been of considerable value was for determining zinc when only extremely small weights of sample were available, *e.g.*, for archeological material, samples weighing 50 mg or less were used in determining zinc contents ranging from 0·001 to 0·2 per cent.

CONCLUSIONS

Atomic-absorption spectrophotometry has been shown to be a rapid and accurate method for determining zinc in metallurgical materials. Inter-element effects are negligible,

and interference caused by molecular absorption from halogen acids is overcome by avoiding the use of such acids. The decreased absorption obtained when relatively concentrated solutions are used is largely accounted for by the reduced efficiency of the atomiser, but this can be overcome by using suitable standard solutions.

TABLE IV

ZINC CONTENTS OF COPPER AND ALUMINIUM ALLOYS BY VARIOUS METHODS

Description of sample	Sample No.	Zinc content found by— polarographic method, %	gravimetric method, %	volumetric method, %	proposed method, %
Alloys containing copper—					
Copper - zinc alloy (99·5% Cu; 0·5% Zn)	—	—	0·51	—	0·50
Copper - zinc alloy (99·0% Cu; 1·0% Zn)	—	—	1·01	—	1·00
Copper - zinc alloy (98·5% Cu; 1·5% Zn)	—	—	1·56	—	1·53
Everdur alloy*	J27	0·89	0·90	—	0·91, 0·91, 0·92
	E422	0·28	0·33	—	0·28, 0·29, 0·30
	E432	0·10	0·11	—	0·097
	349	—	0·32	—	0·32
	416	—	0·16	—	0·18
	417	—	0·048	—	0·044
Phosphor bronze ..	168	0·72	0·72	—	0·72
	158	0·32	0·31	—	0·32
	512	—	0·020	—	0·017
	152	—	0·16	—	0·17
	403	—	0·097	—	0·096
	173	0·058	—	—	0·062
	177	0·27	—	—	0·27
Gilding metal (85% Cu; 15% Zn) ..	—	—	14·8	—	14·8
Alloys containing aluminium—					
Aluminium alloy ..	16	—	0·08	—	0·087, 0·093
	19	—	0·10	—	0·13
	21	—	0·26	—	0·25
	24	—	0·76	—	0·77
	30	—	1·01	—	0·96, 1·02
Copper - aluminium alloy (50% Cu; 50% Al) ..	1422	—	—	0·10, 0·14	0·10
	1427	—	—	0·63, 0·60, 0·64	0·63
	1428	—	—	0·42, 0·44	0·41
	1429	—	—	0·66, 0·67	0·64
Complex alloy	—	—	4·49, 4·37	—	4·44

* This alloy contained copper, silicon and maganese.

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The Determination of Nitrogen in Organic Compounds by the Indanetrione Hydrate Method

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A spectrophotometric method for the determination of ammonia by means of indanetrione hydrate is described. The method has been used for the determination of nitrogen in serum, protein hydrolysates and heterocyclic compounds (*e.g.*, nicotinic acid, 8-hydroxyquinoline and streptomycin) on the micro scale. The reaction between indanetrione hydrate and ammonia has been investigated to determine the optimum conditions for the development of the coloured condensation product of the reaction. The effects of cations on the reaction between ammonia and indanetrione hydrate and the inhibition of the reaction caused by the presence of selenium dioxide in the reaction mixture have been studied. The quantitative determination of nitrogen on an ultra-micro scale is possible by using suitable micro cells in the photometric procedure. Quantitative recoveries have been obtained from 0·2-ml samples of buffered solutions containing 0·5 μg of nitrogen, as ammonia.

THE determination of nitrogen in organic compounds has been performed by either of the well established methods attributed to Dumas and Kjeldahl. Both these methods have been applied on the micro scale, and the presence of approximately 300 μg of nitrogen in the sample is required for an accurate determination.

There have been several modifications of the Dumas technique, including those described by Unterzaucher,[1] Schöniger[2] and Ingram,[3] which were introduced to hasten the combustion process. The Kjeldahl method has been modified by many workers, and a review by Middleton and Stuckey[4] of these modifications indicates the factors in the digestion process that were controlled or modified by each individual investigator. White and Long[5] introduced a method of digesting the nitrogenous sample in sulphuric acid at high temperature. The principle of the rest of the procedure has remained the same in all these modifications of the Kjeldahl method, *viz.*, the distillation and titration of the ammonia in the digested product.

Recently there was reported from this laboratory a method by which the ammonia formed when a nitrogenous compound was digested with sulphuric acid was determined spectrophotometrically by using indanetrione hydrate.[6] This method was first used for the determination of nitrogen in proteins or protein hydrolysates.

METHOD

REAGENTS—

Unless otherwise stated, all materials were of recognised analytical grade. All aqueous solutions were prepared with conductivity water obtained as described previously.[7]

Sodium hydroxide.
Sulphuric acid—Microanalytical-reagent (M.A.R.) grade.
Copper sulphate, anhydrous—M.A.R. grade.
Potassium sulphate—M.A.R. grade.
Selenium.
Mercuric oxide—Laboratory-reagent grade.
Citric acid.
Stannous chloride dihydrate.
Sodium acetate, hydrated.
Glacial acetic acid.
Hydrochloric acid.

Methyl Cellosolve—Methyl Cellosolve (2-methoxyethanol) was redistilled after peroxides had been removed by adding to each litre of solvent 10 ml of a solution prepared by dissolving 50 g of analytical-reagent grade ferrous sulphate heptahydrate in 100 ml of 2·0 *M* sulphuric acid.

Indanetrione hydrate reagent solution—This reagent was prepared by mixing 50 ml of a 4 per cent. stock solution of indanetrione hydrate in methyl Cellosolve with 25 ml each of de-ionised water and sodium acetate buffer solution (pH 5·5) and 0·08 g of stannous chloride. The 4 per cent. stock solution of indanetrione hydrate was prepared as described previously.[8]

Sodium citrate buffer solution, 0·4 M, pH 5·0—Four hundred millilitres of *N* sodium hydroxide and 42·0 g of citric acid dissolved in 1 litre.

Sodium acetate buffer solution, 4·0 M, pH 5·5—Hydrated sodium acetate (544 g) was dissolved in 500 ml of water, with heating on a water bath. The solution was cooled, 100 ml of glacial acetic acid were added, and the mixture was diluted to 1 litre.

Catalyst—The catalyst used with the concentrated sulphuric acid in the digestion of samples consisted of a mixture of potassium sulphate, copper sulphate, mercuric oxide and selenium in the proportions 15:5:5:1, respectively.

APPARATUS—

Small volumes of samples (0·010 to 0·500 ml) were dispensed by means of an Agla micrometer syringe (Burroughs Wellcome Ltd., London); larger volumes of solutions were delivered from British Standard pipettes (grade A).

The photometric procedure was similar to that described previously,[8] except that a Uvispek spectrophotometer (with rectangular cells of path length 1 cm) was used in place of a Unicam SP350 spectrophotometer.

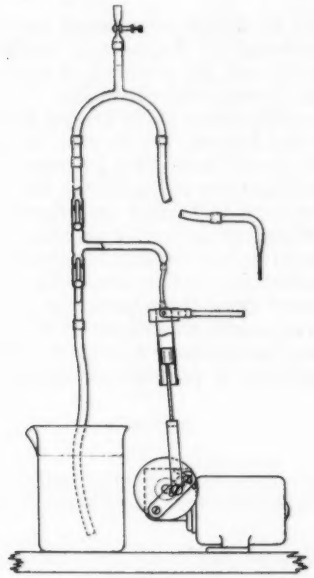

Fig. 1. Automatic dispenser

An automatic dispenser, shown in Fig. 1, was used to add the volume of ethanol - water diluent (usually 6·0 ml) to the reaction mixture of sample and indanetrione hydrate. The dispenser consisted of a graduated cylindrical glass tube (the barrel of a standard 10-ml hypodermic syringe) connected by plastic tubing to a two-way glass valve. The inlet tube of the valve was connected by plastic tubing to the pool of ethanol - water diluent, and the outlet tube of the valve was used to direct a fixed volume of ethanol - water mixture (6·0 ml) into the contents of a test-tube (usually 4·0 ml) after reaction between the sample and the indanetrione reagent. A metal piston in the graduated cylinder was attached to an adjustable eccentric driven by an electric motor through a reduction gear. (The glass valve was obtained from A. and T. Glass Components Ltd., 50 Farnby Avenue, Stanmore, Middlesex.)

The digestion of a heterocyclic compound in sulphuric acid at high temperature was completed in a sealed Pyrex-glass ampoule. The ampoule was placed inside a brass container, as shown in Fig. 2, and this in turn was placed inside a wrought-iron cylinder with threaded cap. The over-all dimensions of the brass container and wrought-iron cylinder were 15·5 and 34·0 cm, respectively. The concentric brass tubes forming the container had a wall thickness of 1·0 mm, and there was a hole 1·0 mm in diameter through the wall near the end of the wider tube. The wrought-iron cylinder had a wall thickness of 3 mm, and the threaded cap had a hole 2 mm in diameter through its centre. Two brass containers, one to contain the sealed ampoule with sulphuric acid, catalyst and sample and the other to contain another sealed ampoule with sulphuric acid and catalyst, were heated together in the wrought-iron cylinder.

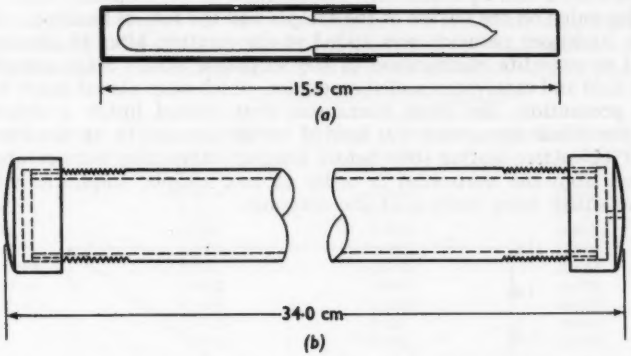

Fig. 2. (*a*) Ampoule in brass container; (*b*) wrought-iron cylinder

The excess of sulphuric acid present in the combustion flask or ampoule after digestion of the sample was neutralised by the addition of a concentrated solution of sodium hydroxide. The volumes of sulphuric acid (0·25 ml) and concentrated solution of sodium hydroxide (approximately 0·6 ml) were dispensed from the all-glass precision pipettes shown in Fig. 3. One precision pipette, see Fig. 3 (*a*), had a maximum capacity of 0·250 ml and was made by fusing a calibrated length of precision-bore Veridia-glass tubing (obtained from Chance Bros., Birmingham) to the barrel of a standard 1-ml hypodermic syringe. The second precision pipette, see Fig. 3 (*b*), was constructed by fusing a Pyrex-glass NPL calibrated 1·0-ml pipette to the barrel of a Pyrex-glass standard 2-ml hypodermic syringe.

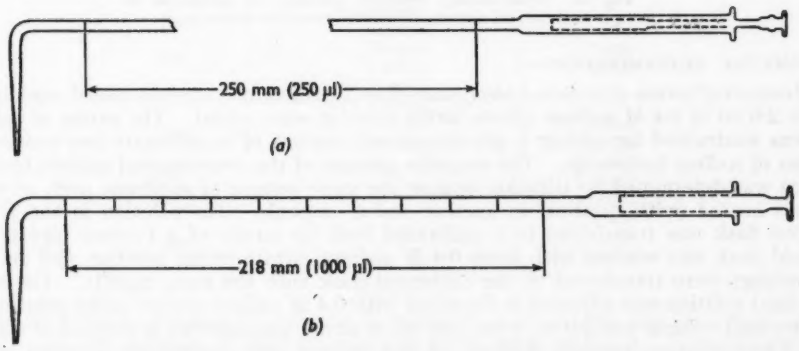

Fig. 3. Precision pipettes of capacity (*a*) 0·25 ml and (*b*) 1·00 ml

The quartz cells used in the photometric determination of ultra-micro amounts of ammonia had a path length of 10 mm, a width of 2·3 mm and a capacity of approximately 0·5 ml.

PROCEDURE FOR DIGESTING SAMPLE—

Samples of serum (0·01 to 0·10 ml) were measured from an Agla syringe, samples of nicotinic acid, 8-hydroxyquinoline and methionine were transferred to Pyrex-glass 5·0-ml ampoules by means of a weighing stick, and all solid samples were weighed by means of an Oertling microbalance (model 142). The requisite volume (0·25 ml) of M.A.R. grade sulphuric acid and 2 to 3 mg of catalyst were added to the sample, which was then digested. The digestion was completed in an ordinary micro Kjeldahl flask (for serum or protein hydrolysate) or a sealed Pyrex-glass ampoule (for solid samples). The serum or protein hydrolysate was digested for between 2 and 2½ hours after clarification of the sulphuric acid. This period of "after-boil"[4] depended on the nature of the sample and the rate of heating. A 0·2-ml portion of 30 per cent. hydrogen peroxide was added to the mixture after 45 minutes of the initial heating period to expedite clarification of the sulphuric acid. Solid samples were heated with sulphuric acid and catalyst in sealed ampoules, which were placed inside brass containers. As an added precaution, the brass containers were placed inside a closed wrought-iron cylinder, and the whole apparatus was heated for 30 minutes in an electric muffle furnace at 460° to 480° C. After sealing (but before heating), ampoules were rotated in a position slightly inclined from the horizontal in order to mix sample, sulphuric acid and catalyst and to wet the entire inner surface of the ampoule.

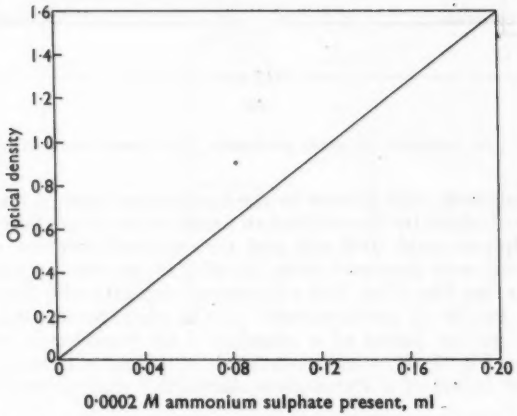

Fig. 4. Relationship between amount of ammonia in sample and optical density of diluted reaction mixture

PHOTOMETRIC DETERMINATION—

Analysis of serum or protein hydrolysate—The digestion mixture was cooled, and approximately 2·0 ml of 0·4 *M* sodium citrate buffer solution were added. The excess of sulphuric acid was neutralised by adding a pre-determined amount of a carbonate-free concentrated solution of sodium hydroxide. The requisite amount of the concentrated sodium hydroxide solution was determined by titration against the same volume of sulphuric acid, as control, and was heated (with catalyst) in another sealed ampoule. The solution in the Kjeldahl digestion flask was transferred to a calibrated flask by means of a Pasteur pipette. The Kjeldahl flask was washed with fresh 0·4 *M* sodium citrate buffer solution (pH 5·0), and the washings were transferred to the calibrated flask with the same pipette. The volume of the final solution was adjusted to the mark with 0·4 *M* sodium citrate buffer solution.

The final volume was 5·0 ml when 0·01 ml of serum was digested in 0·25 ml of sulphuric acid. Three aliquots (usually 0·25 ml) of this diluted and neutralised digestion mixture were each diluted to 2·0 ml with the same buffer solution. Each of these three 2·0-ml samples

was mixed with 2·0 ml of a 2 per cent. solution of indanetrione hydrate reagent. The reaction mixtures were placed in separate test-tubes, and the test-tubes were covered with metal caps and immersed in a water bath at 100° C for 30 minutes. The reaction mixtures were then cooled, and each (4·0 ml) was diluted with 6·0 ml of ethanol - water mixture (1 + 1 v/v). The optical density of each diluted reaction mixture was measured in a 1-cm cell at 570 mμ with a Uvispek spectrophotometer. A 2·0-ml sample containing 0·4 μ*M* ammonia (equivalent to 5·6 μg of nitrogen) produced 10 ml of diluted reaction mixture having an optical density of 0·805 ± 0·008 when determined under these conditions. The determination of ultra-micro amounts of ammonia was possible by using one-tenth of the volumes of sample, indanetrione hydrate reagent solution and ethanol - water mixture. By this procedure, a determination of 0·56 μg of nitrogen in an 0·2-ml aliquot of solution could be made with the same accuracy as that obtained by the micro method.[6]

TABLE I

EFFECTS OF VARIOUS CATIONS ON OPTICAL DENSITY OF DILUTED REACTION MIXTURE

Each result is the mean of triplicate determinations on a reaction mixture containing 0·4 μ*M* ammonia. The optical density in absence of added cations was 0·804

Element added	Optical density in presence of— 50 μg of element	100 μg of element	500 μg of element
Iron	0·800	0·806	0·802
Zinc	0·807	0·805	0·803
Lead	0·809	0·805	0·800
Copper	0·801	0·800	0·799
Calcium	0·799	0·802	0·805
Barium	0·808	0·803	0·800
Aluminium ..	0·805	0·803	0·801
Magnesium ..	0·807	0·802	0·802
Manganese ..	0·800	0·809	0·801
Cobalt	0·805	0·801	0·800
Nickel	0·800	0·805	0·806

TABLE II

EFFECTS OF SELENIUM AND MERCURY ON OPTICAL DENSITY OF DILUTED REACTION MIXTURE

Amount of element present, μg	Optical density in presence of selenium	Optical density in presence of mercury
Nil	0·808	0·805
	0·807	0·802
	0·812	0·808
25	0·804	0·810
	0·811	0·808
50	0·809	0·802
	0·811	0·810
75	0·811	0·808
	0·807	0·808
100	0·809	0·808
	0·802	0·814
125	0·811	—
	0·804	—
150	0·812	0·808
	0·814	0·807
175	0·802	—
	0·807	—
200	0·789	0·808
	0·794	0·808
250	—	0·804
		0·802
300	—	0·805
		0·808
500	—	0·804
		0·808

RESULTS

The relationship between the amount of ammonia in the sample and the optical density of the final diluted reaction mixture (normally 10 ml) is shown in Fig. 4. The optical density of the solution was found to be directly proportional to the amount of ammonia present in

the sample, even for optical-density values in the region of 1·6. Ultra-micro amounts of ammonia (0·01 to 0·04 μM) could be determined with the same accuracy when the volumes of sample, indanetrione hydrate solution and ethanol - water mixture were dispensed from an Agla syringe and precision pipettes.

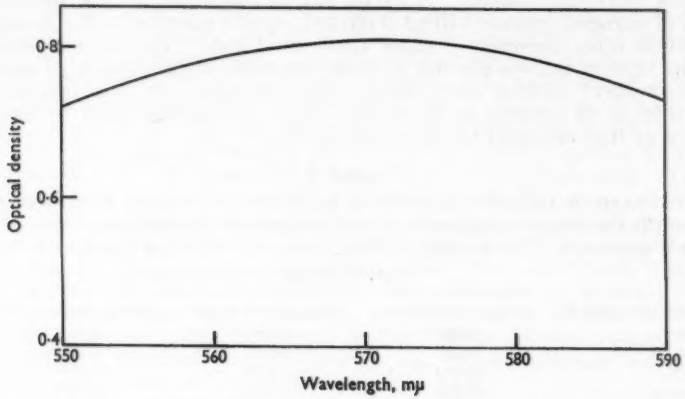

Fig. 5. Relationship between wavelength of absorbed radiation and optical density of diluted reaction mixture containing 0·4 μM ammonia

The effects of added cations on the optical density of a diluted reaction mixture containing 0·4 μM ammonia (equivalent to 5·6 μg of nitrogen) are shown in Table I; the results in Table II show the inhibition of the reaction between ammonia and indanetrione hydrate in presence of selenium dioxide.

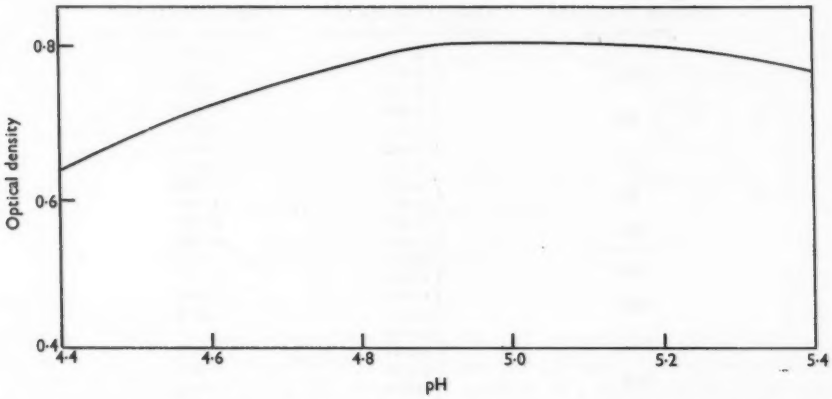

Fig. 6. Relationship between pH of reaction mixture and optical density of diluted reaction mixture containing 0·4 μM ammonia

The absorption curve shown in Fig. 5 indicates the relationship between the wavelength of absorbed radiation and the optical density of a solution resulting from the reaction between indanetrione hydrate reagent solution and 0·4 μM ammonia under the standard conditions already described.

The relationship between the optical density and pH of the reaction mixture for samples containing 0·4 μM ammonia is shown in Fig. 6.

TABLE III

COMPARISON OF RESULTS FOR NITROGEN BY PROPOSED AND KJELDAHL METHODS

Sample	Nitrogen found by proposed method, mg per ml	Nitrogen found by Kjeldahl method, mg per ml
Normal horse serum (0·01 ml)	11·0	10·9
	10·9	11·0
	10·9	11·0
Normal rat serum (0·1 ml)	11·3	11·4
	11·3	11·3
	11·4	11·3
Alkaline collagen hydrolysate	0·618	0·615
	0·610	—
	0·616	—
Gyoglobulin hydrolysate	0·512	0·509
	0·511	—
Penicillinase 5B	0·548	0·544
	0·545	—
Prepared mixture of amino acids	0·885	0·885
	0·892	—
	0·889	—

In Table III, results for the determination of nitrogen in sera and protein hydrolysates by the indanetrione method are compared with those obtained by the standard micro Kjeldahl method (Pregl technique).

The results for the determination of nitrogen in heterocyclic compounds, *viz.*, nicotinic acid, 8-hydroxyquinoline, streptomycin and methionine, are shown in Table IV. Methionine was analysed after digestion in a sealed ampoule at different temperatures.

TABLE IV

NITROGEN CONTENTS OF VARIOUS ORGANIC COMPOUNDS

Sample	Amount of sample taken, mg	Nitrogen content found, %	Theoretical nitrogen content, %
Nicotinic acid	0·566	11·4	11·39
	0·567	11·3	
	0·615	11·3	
	0·541	11·3	
	0·705	11·2	
	0·467	11·5	
Methionine	0·922	9·2	9·39
	0·719	9·6	
	0·623	9·4	
	0·486	9·4	
8-Hydroxyquinoline	0·430	9·6	9·65
	0·660	9·6	
Streptomycin	0·815	13·3	13·45
	0·815	13·3	
	0·815	13·5	

DISCUSSION OF RESULTS

Fig. 4 shows that nitrogen, as ammonia, can be quantitatively determined on the micro and ultra-micro scales. The accuracy of the method when applied to serum is dependent on careful control of the digestion of the sample and adjustment of the pH of the reaction mixture. The digestion of solid samples with sulphuric acid at high temperature in a sealed ampoule must be controlled within certain temperature limits. Conditions were satisfactory when the temperature of the furnace was between 460° and 480° C; loss of nitrogen was recorded when the temperature of the furnace was 560° C.

The determination of nitrogen in methionine was performed on samples digested at different high temperatures. The experiments showed that this compound, which could be determined directly by means of the indanetrione reagent, was analysed successfully when the temperature of the digestion with sulphuric acid did not exceed 480° C.

The results in Table I indicate that, after removal of cations from the 4 per cent. stock solution of indanetrione reagent by the treatment already described,[8] the presence of 500 μg of aluminium, calcium, magnesium, barium, iron, zinc, lead, copper, manganese, cobalt or nickel did not inhibit the reaction between ammonia and indanetrione hydrate.

Selenium, as dioxide, did not affect the colour yield of the complex formed by the reaction between ammonia and indanetrione hydrate (see Table II) unless present in amounts exceeding 175 μg in a 2·0-ml sample (micro method). The amount of selenium in the normal aliquot taken for analysis was between 5 and 10 μg, and this had no effect on the colour yield. Similarly, the presence of mercury in the catalyst used with sulphuric acid for digesting the sample had no influence on the colour. Each aliquot, containing approximately 30 μg of mercury, gave the same colour yield as that obtained when the equivalent amount of ammonia was allowed to react in absence of mercury.

It has been shown[9,10] that, provided the 4 per cent. stock solution of indanetrione hydrate in methyl Cellosolve is treated with a cation-exchange resin, the colour yield from a standard amount of α-amino acid is consistent, whatever the source of supply of the reagent. In this work the quantitative determination of ammonia has been facilitated in the same manner. Selenium, probably as selenite, is known to be present in commercial samples of indanetrione hydrate to a varying degree. The treatment of the stock solution of indanetrione hydrate with a cation-exchange resin avoids the use of ethylenediaminetetra-acetic acid[11] in the reaction mixture. In presence of stannous chloride as reducing agent, the indanetrione hydrate reacts with ammonia to form a complex having an absorption maximum not affected by the presence of relatively large amounts of cations (see also paper by Meyer and Riklis[12]). The proposed method requires a weight of sample approximately one-fifth of that needed for the determination of nitrogen by the micro Dumas method. For streptomycin, the indanetrione method gave quantitative recoveries, whereas the micro Kjeldahl method was unsatisfactory and the Dumas method yielded inconsistent and low recoveries.

I thank Mr. C. Couling for preparing Figs. 1 and 2 and Mr. R. A. Faulkes for valuable technical assistance.

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The Determination of Ketone Bodies in Blood

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A method is described for the accurate quantitative determination of ketone bodies in blood. The method is based on the conversion of all ketone bodies to acetone, distillation of acetone and its subsequent colorimetric determination by reaction with ethanolic salicylic aldehyde to form dihydroxydibenzene acetone in alkaline solution. Losses of acetone during distillation are readily avoided, and free acetone *plus* acetoacetic acid are determined separately from β-hydroxybutyric acid in the same sample. The method is reliable at low blood ketone levels. For each ketone fraction the standard deviation of a single determination is less than ±0·15 mg per 100 ml of blood at levels below 3 mg per 100 ml. The percentage error does not change appreciably at higher levels. With little reduction in accuracy, the method can be adapted for determining all ketone bodies in a single distillate.

In most methods for determining ketone bodies, acetoacetic acid and β-hydroxybutyric acid are converted to acetone for determination. If this is effected during distillation, preformed acetone *plus* acetoacetic acid can be determined separately from β-hydroxybutyric acid. The principal criticisms of this technique are that acetone may be lost during distillation[1] and that changes in the concentration of the acid dichromate reagent during distillation lead to variable errors in the determination of β-hydroxybutyric acid.[2] If the conversion to acetone is effected by heating under reflux or in an autoclave, β-hydroxybutyric acid recoveries are higher and are reported to be more reproducible; however, recoveries of acetoacetic acid may be low,[3,4,5] and it is not possible to determine pre-formed acetone *plus* acetoacetic acid separately from β-hydroxybutyric acid in the same sample. Greenberg and Lester[6] and Thin and Robertson[7] have proposed methods involving an empirical step in the removal of apparently unknown interfering substances.

The method recently proposed by Mayes and Robson[8] includes the advantages of both procedures by using a combined reflux and distillation apparatus. However, as with many other methods, their procedure is time-consuming unless many sets of apparatus are available.

In studies on the pathogenesis of bovine ketosis at the Veterinary Laboratory of the Ministry of Agriculture and Fisheries, Weybridge, England, during the 1930's, methods then existing for the determination of ketone bodies in blood were found to be too inaccurate and insensitive at low levels (less than 5 mg of acetone per 100 ml of blood) for precise work. Under the guidance of Dr. H. H. Green and with the collaboration of Dr. A. Eden, a micro colorimetric method was evolved. Based on the method of Behre and Benedict,[9] this method was first referred to by Eden and Green,[10] but, for various reasons associated with the transfer of Dr. Eden to another laboratory and the later retirement of Dr. Green, has not been published in full.

We have modified and improved this method in several important respects. The method is now convenient in simplicity of equipment and is extremely sensitive and rapid; its accuracy is sufficient for all purposes. The results presented are solely the outcome of our investigations over the last 8 years.

Method

Apparatus—

The distillation apparatus in use in this laboratory is shown in Fig. 1. Advantage is taken of the fact that it is easily possible to obtain all the acetone in the first few millilitres of distillate when dealing with volumes as large as 25 ml, by designing the apparatus to act to some extent as a fractionating column. The distilling tube is 200 mm × 25 mm. The air-condenser tubing is of 5 mm internal diameter, the portion sloping upwards at an angle of 45° being 200 mm long; the water-condenser tubing is of 3·5 mm internal diameter. The blood filtrate and, later, the dichromate solution are delivered from a dropping funnel through a capillary tube. This serves two purposes. First, it controls the delivery rate, which is constant from one distillation to another, so that the stopcock on the dropping funnel

can be fully opened. Secondly, loss of acetone does not occur if the stopcock is not closed immediately on completion of delivery, because the small amount of solution remaining in the end of the capillary is not forced back up the capillary tube by the pressure inside the boiling tube. These features greatly assist the convenience of the operation. Capillary tubing of internal diameter approximately 0·25 mm allows a dropping rate of not less than 5 ml per minute and satisfies the requirements that the rate of addition should not be so fast that boiling is interrupted or so slow that the solutions will not run freely against the pressure inside the boiling tube.

Bumping, particularly when the distilling solution first boils, is completely prevented by the use of a suitable ebullition tube. This is readily made from tubing of approximately 5 mm external diameter; the tubing is sealed about 5 mm from the bottom end and also at the top; to facilitate removal from the boiling tube it should be at least 125 mm long.

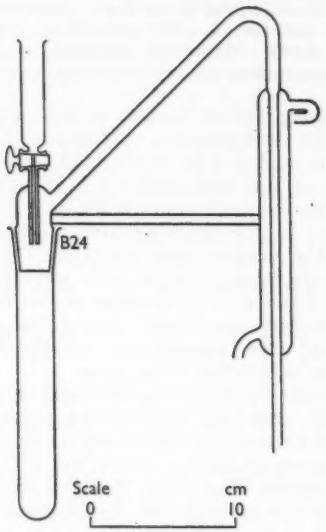

Fig. 1. Distillation apparatus

REAGENTS—

De-proteinisation solutions—A 5 per cent. w/v solution of zinc sulphate, $ZnSO_4.7H_2O$, and 0·3 *N* barium hydroxide. The protein-precipitation technique used is that proposed by Somogyi.[11] Absolute accuracy in the preparations of these solutions is not so important as the requirement that a given volume of barium hydroxide shall exactly neutralise an equal volume of zinc sulphate.

Sulphuric acid, 18 N *and* 7 N.

Potassium dichromate solution, 0·2 *per cent. w/v.*

Sodium hydroxide, 10 N.

Ethanol, redistilled—Acetone-free.

Salicylic aldehyde—British Drug Houses Ltd. laboratory-reagent grade salicylic aldehyde was used.

Colour reagent solution—Ethanolic salicylic aldehyde prepared by diluting 20 ml of salicylic aldehyde to 100 ml with acetone-free ethanol; the solution was stored in a refrigerator.

Standard solutions—Standard aqueous solutions of redistilled analytical-reagent grade acetone containing 20 mg of acetone per 100 ml are stable for long periods in a refrigerator. Standard solutions of sodium acetoacetate were prepared from recently distilled ethyl acetoacetate by Krebs and Eggleston's method[12]; they are stable for several weeks when refrigerated. Aqueous solutions of sodium β-hydroxybutyrate (obtainable from the British Drug Houses Ltd. or L. Light & Co. Ltd.) show no deterioration after 2 months at 0° C.

PROCEDURE—

De-proteinisation—Prepare protein-free filtrates from whole blood at dilutions of 1 in 10 to 1 in 80, depending on the blood ketone level, by Somogyi's method.[11]

Distillation: Acetoacetic acid and pre-formed acetone—Place 8 ml of 7 *N* sulphuric acid in the distilling tube, containing an ebullition tube, and begin distillation. When distillate begins to run from the condenser (but not until then), add to the contents of the distilling tube from the dropping funnel a 5-ml sample of blood filtrate measured by pipette; boiling should not be interrupted. Continue distillation at a steady rate until 5 ml of distillate have been collected in approximately 5 minutes in a 150-mm × 15-mm test-tube calibrated accurately at the 5-ml level. If more than three distillations are being carried out simultaneously, it may be advisable to distil slightly less than 5 ml, subsequently making up to the mark with distilled water.

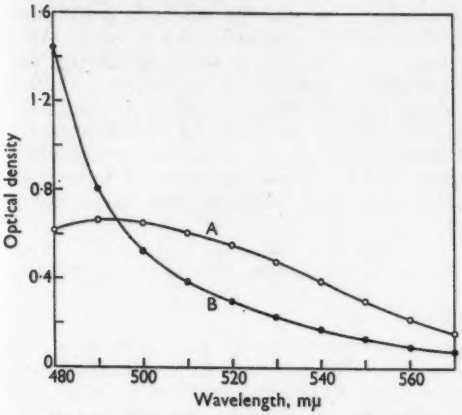

Fig. 2. Absorption curves: curve A, acetone standard containing 0·4 mg of acetone per 100 ml (measured against reagent blank); curve B, reagent blank (measured against distilled water)

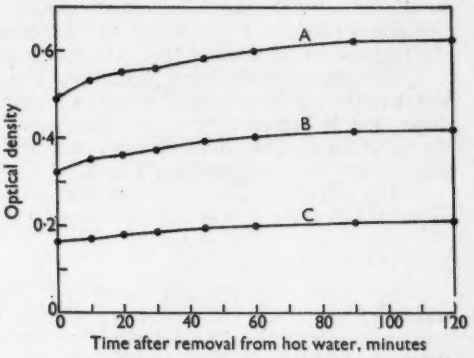

Fig. 3. Colour development in aqueous acetone solution during cooling after reaction with alkaline salicylic aldehyde and 20 minutes' incubation at 55° C; curve A, 0·6 mg of acetone per 100 ml; curve B, 0·4 mg of acetone per 100 ml; curve C, 0·2 mg of acetone per 100 ml

Distillation: β-Hydroxybutyric acid—Remove the collection tube, containing pre-formed acetone and acetone formed by decarboxylation of acetoacetic acid, and place a fresh tube in position without interrupting the boiling. Run in 5 ml of potassium dichromate solution, previously placed in the dropping funnel, and continue distillation until 5 ml of distillate containing acetone from the oxidation of β-hydroxybutyric acid have been collected. Pass distilled water through the dropping funnel to remove traces of dichromate solution from the capillary tube before beginning the next distillation.

If boiling is interrupted between distillations, the ebullition tube must be shaken to replace with air the solution trapped in its lower end. If this procedure is omitted, serious bumping, even of explosive violence, may occur when boiling is next begun.

Colour development—Adjust the volumes of distillates to 5 ml (if necessary), and add 4 ml of 10 *N* sodium hydroxide and 2 ml of colour reagent solution to each; mix during addition of the latter. Shake the tubes vigorously (a surgeon's finger cot can be used to protect the thumb against alkali), and place in a water bath at approximately 55° C for 20 minutes. Remove, and set aside to cool at room temperature for 1 hour. Measure the colour developed against a reagent blank solution, prepared at the same time in 5 ml of distilled water, with a spectrophotometer at 530 mμ in 10-mm cells.

Standards—Standards run with each series of determinations should include duplicate undistilled acetone standards (usually 0·5 mg per 100 ml) not previously taken through the protein-precipitation procedure and a mixed acetoacetic acid or acetone and β-hydroxybutyric acid solution (usually 5 mg of each, as acetone, per 100 ml), the latter being taken through the entire procedure, with a single protein precipitation and triplicate distillations.

We prefer a mixed standard containing acetone rather than acetoacetic acid, because losses before and during distillation, if they occur, are greater and hence more readily detectable.

Results

All results were obtained on a Unicam SP600 spectrophotometer.

Colorimetric determination of acetone—

Absorption curves recorded on the straw-coloured reagent blank solution (measured against distilled water) and on a solution containing 0·4 mg of acetone per 100 ml (measured against the reagent blank) are shown in Fig. 2. In selecting a suitable wavelength, some compromise is necessary to avoid high blank readings and yet to maintain an appreciable sensitivity; 530 mμ was chosen as a suitable compromise.

The blank reading should be checked for each batch of salicylic aldehyde before use; if it is less than 0·200 when measured against distilled water, no purification is needed. After purification by standard procedures, blank readings were as low as 0·050 at 530 mμ and permitted readings to be made at shorter wavelengths. However, it is doubtful whether the increase in an already high sensitivity is necessary or the steps to achieve it justifiable.

Maximum colour development was not reached, even 2 hours after removal from the water bath; for all practical purposes, however, readings can be made at 60 or 90 minutes, depending on the number of determinations being carried out simultaneously and hence on the significance of the small changes in optical density that occur during the interval necessary to complete the readings (see Fig. 3).

Beer's law was always strictly obeyed over the range 0 to 1·2 mg of acetone per 100 ml of blood filtrate and was usually obeyed over the range 0 to 1·6 mg of acetone per 100 ml.

Distillation losses—

Acetoacetic acid and free acetone—If the procedure recommended for acetoacetic acid and free acetone was followed, but with addition of blood filtrate to 7 *N* sulphuric acid before heating was begun, recoveries of acetoacetic acid were of the order of 80 to 90 per cent. and those of acetone could be as low as 70 per cent. This loss was due to the rapidity of decarboxylation of acetoacetic acid, to the low boiling-point of acetone and to the low ratio between the volumes of the condensers and distillation tube. During the initial heating and in the early stages of boiling before steam began to condense, acetone vapour was forced out with the expanding air. This loss could be almost completely avoided by careful control of the initial rate of heating; this is tedious and leads too easily to personal errors and hence to a reduction in reproducibility of results.

Addition of the blood filtrate from the dropping funnel after boiling had begun and equilibrium had been reached in the condenser system completely avoided loss of acetone without the need to vary the rate of heating during the distillation; as many as six distillations could then be carried out simultaneously.

Blank determinations on distilled water taken through the entire procedure, including protein precipitation, did not differ significantly from undistilled acetone blanks.

Table I

Recoveries from standard solutions of acetone, acetoacetic acid and β-hydroxybutyric acid at various concentrations

All concentrations expressed as acetone

	Concentration, mg per 100 ml	Number of determinations	Mean recovery, %	Standard deviation, %	Standard deviation, mg per 100 ml
Acetone	2 to 3	18	100·4	5·2	0·13
	4 to 6	25	100·2	3·6	0·18
	7 to 10	24	100·7	4·1	0·33
Acetoacetic acid	2 to 5	25	101·2	4·3	0·16
β-Hydroxybutyric acid	2 to 3	21	75·2	6·0	0·13
	4 to 6	21	76·1	4·1	0·21
	7 to 12	25	74·7	4·1	0·34

Recoveries of acetone from aqueous solutions averaged 98 to 102 per cent. at levels of 2 to 10 mg per 100 ml. From time to time the errors of different analysts have been checked by carrying out 12 to 18 determinations on the same solution. Mean recoveries on five occasions, involving two analysts, varied between 98·3 and 101·4 per cent. The standard deviation of a single determination never exceeded 0·22 mg per 100 ml at a concentration of 5 mg of acetone per 100 ml. Similar recoveries and errors were obtained with solutions of acetoacetic acid.

On several occasions duplicate or triplicate determinations were carried out on solutions of various concentrations; the results are summarised in Table I. Recovery did not vary with concentration, but the absolute error was least at low concentrations.

In one period of 6 months, thirty-two series of blood analyses were carried out on different days. Mean recoveries from duplicate acetone standards on these days varied from 95·5 to 103·5 per cent., the over-all mean being 99·6 per cent. In another similar period, mean recoveries from 19 duplicate standards ranged from 94 to 102 per cent., the over-all mean being 98·1 per cent.

Ethyl acetoacetate has proved an unsatisfactory standard for our method. On six occasions when recoveries from sodium acetoacetate standards were 100 $\pm$ 2 per cent., recoveries from ethyl acetoacetate standards varied from 77 to 93 per cent.

β-Hydroxybutyric acid—With the procedure recommended, recoveries of acetone from β-hydroxybutyric acid averaged 72 to 76 per cent. of the theoretical. The errors of different analysts have been checked in the same way as for acetone. Mean recoveries on eight occasions, involving four analysts, varied between 71·7 and 75·5 per cent. The standard deviation of a single determination never exceeded 0·31 mg per 100 ml at a level of 5 mg, as acetone, per 100 ml.

As with acetone and acetoacetic acid, duplicate or triplicate determinations were carried out on several occasions on solutions of various concentrations; the results are summarised in Table I. Recovery did not vary with concentration, but, as with acetone, the absolute error was least at low concentrations.

As oxidation and distillation proceed simultaneously, the concentration of reagents is continuously changing. Mayes and Robson[8] have claimed that the reagents must remain at constant concentrations throughout the period of oxidation if reproducible results in the determination of β-hydroxybutyric acid are to be ensured. Accordingly, distillations were carried out in which the rate of boiling was reduced to an extent sufficient to ensure that only a few drops of distillate were collected in 15 minutes. After this period of "heating under reflux," distillation was carried out at the normal rate, so that 5 ml of distillate were collected in a further 5 minutes. Recovery was increased to 80 per cent., compared with 85 to 86 per cent. obtained by Weichselbaum and Somogyi,[2] Greenberg and Lester[6] and Mayes and Robson,[8] who used other procedures involving heating under reflux. However, the error was no less than that obtained by the same analyst with the shorter proposed method.

Most methods for determining β-hydroxybutyric acid give less than 100 per cent. of the theoretical recovery of acetone; all must depend on rigid standardisation of procedure if high reproducibility is to be obtained. As long as this principle is followed, there is no special virtue in prolonging a procedure to increase recoveries to an arbitrarily accepted maximum of 85 per cent. It should be pointed out that distillation times vary little in this laboratory during a series of distillations with butane gas at constant pressure; variable gas pressures at different times of day associated with large town supplies are known to lead to considerable variations in distillation times.

Determination of total bodies in a single distillate

As recovery of acetone from β-hydroxybutyric acid is not complete, the determination of total ketone bodies in a single distillation involves an assumption of the proportion of total ketone bodies that this acid forms. This proportion may vary considerably from normal in man[8] and in ruminants[13]; we have observed ratios of acetone *plus* acetoacetic acid to β-hydroxybutyric acid varying from 1 to 1 to 1 to 11 in ewes with ketosis.

In sheep, as in man, the normal ratio is near 1 to 2. If total ketone bodies are determined in a single distillation and this ratio is assumed, it can be calculated that the maximum error of the estimate is $\pm$5 per cent. if the true ratio lies between 1 to 1 and 1 to 5, increasing to −9 per cent. as the true ratio increases to 1 to 15.

The simplest technique for determining both acetoacetic acid and β-hydroxybutyric acid in a single distillation would appear to be addition of filtrate to boiling 7 *N* sulphuric acid containing potassium dichromate, and then distillation for 5 minutes. However, the conversion of acetoacetic and β-hydroxybutyric acids to acetone by heating directly in acid dichromate solution, as proposed by Greenberg and Lester[6] and Thin and Robertson,[7] has been criticised by several workers. In our hands, this procedure gave recoveries of only 60 per cent. of the theoretical amount of acetone from acetoacetic acid, 48 per cent. from ethyl acetoacetate and 60 to 65 per cent. from β-hydroxybutyric acid, at levels of 3 to 12 mg. as acetone, per 100 ml. In this respect, our results for acetoacetic acid agree with those of Michaels *et al.*,[3] Werk *et al.*[4] and Hird and Symons[14] and for β-hydroxybutyric acid with those of Thin and Robertson.[7]

The method described below was finally adopted for use when only a total ketone figure was required and when a high order of accuracy was not needed. A 5-ml sample of blood filtrate was placed in the distilling tube with 3 ml of 18 *N* sulphuric acid. Heating was begun at the usual rate and was continued until collection of distillate had begun. Potassium dichromate solution (5 ml) was then introduced from the dropping funnel, and heating was continued until 5 ml of distillate had been collected in 5 minutes. Colour development was carried out in the usual way.

Recoveries of acetone from acetoacetic acid varied from 80 to 90 per cent. Recovery of acetone from β-hydroxybutyric acid was 72 to 75 per cent., as in the two-stage procedure. Unless pre-formed acetone constitutes an appreciable proportion of the acetone *plus* acetoacetic acid fraction, the maximum additional error likely to be introduced by the assumption of an 85 per cent. recovery of acetone from acetoacetic acid is less than 0·1 mg per 100 ml.

Application of the method to analysis of blood

Recovery experiments have been carried out at various times with solutions of acetone and with mixed solutions of acetoacetic acid and β-hydroxybutyric acid added to blood. Recoveries of acetone were 98 to 101 per cent. when the solutions were added to blood and carried through the protein-precipitation procedure[11] at dilutions of 1 in 10 during protein precipitation. We have observed, however, that centrifugation after protein precipitation by this method may be associated with a loss of acetone of the order of 15 per cent. at a concentration of 5 mg per 100 ml of blood.

Table II

Recoveries of acetoacetic acid and β-hydroxybutyric acid added to blood

		Acetoacetic acid				β-Hydroxybutyric acid			
Date	Dilution during protein precipitation	Added, as acetone, mg per 100 ml	Recovered, as acetone, mg per 100 ml	Mean recovery, %	Number of determinations	Added, as acetone, mg per 100 ml	Recovered, as acetone, mg per 100 ml	Mean* recovery, %	Number of determinations
Protein precipitation with acidified 1·25 per cent. w/v solution of zinc sulphate and 0·75 N sodium hydroxide[15]—									
9.12.58	1 in 20	1·0	0·70	70	8	1·0	0·74	74	8
10.12.58	1 in 10	2·5	2·25	90	3	2·5	2·10	84	3
28. 5.58	1 in 10	2·9	2·55	88	4	2·4	1·70	71	5
29. 5.58	1 in 10	2·4	2·16	90	4	2·0	1·44	72	4
29. 5.58	1 in 10	4·9	4·61	94	2	4·0	3·08	77	2
29. 5.58	1 in 10	7·8	7·41	95	2	8·0	6·96	87	2
Protein precipitation with 5 per cent. w/v solution of zinc sulphate and 0·3 N barium hydroxide[11]—									
4.11.57	1 in 10	—	—	—	—	5·0	4·15	83	9
6.11.57	1 in 10	—	—	—	—	5·0	4·20	84	6
7.11.57	1 in 10	—	—	—	—	3·0	2·55	85	6
6. 6.58	1 in 10	2·4	2·21	92	3	2·0	1·54	77	3
14. 7.58	1 in 80	40·5	40·50	100	5	39·5	38·30	97	6
16. 7.58	1 in 80	20·3	20·30	100	6	19·8	19·50	102	6
17. 7.58	1 in 40	20·3	20·50	101	6	19·8	19·50	102	6
25. 7.58	1 in 10	4·9	4·80	98	6	4·7	4·55	97	6
15.12.58	1 in 20	10·0	9·70	97	6	10·0	9·70	97	6
16.12.58	1 in 20	—	—	—	—	10·0	9·90	99	6
17.12.58	1 in 10	5·0	5·20	104	6	5·0	5·00	100	6
18.12.58	1 in 10	2·5	2·50	100	6	—	—	—	—

* Corrected for losses during simultaneous distillation of standard solution alone.

Recoveries of acetoacetic acid and β-hydroxybutyric acid are summarised in Table II. Complete recovery was never attained by the earlier protein-precipitation technique,[15] in which the reagents are an acidified 1·25 per cent. w/v solution of zinc sulphate and 0·75 *N* sodium hydroxide. Recoveries of acetoacetic acid were higher than those of β-hydroxybutyric acid. The absolute amounts lost were not constant at different concentrations, but the percentage loss was least at the highest concentrations used.

Recoveries were likewise low with one batch of reagents in use between November 1957 and June 1958 for protein precipitation by the later method of Somogyi.[11] However, two subsequent batches of reagents from a different source gave complete recovery of both acids. The reason for this difference is not known.

Discussion of the method

The original objection to distillation techniques for determining acetone, *viz.*, the readiness with which distillation losses occur, has been completely overcome in our method, as it also has in that proposed by Mayes and Robson.[8]

For each ketone fraction the standard deviation of a single determination was less than ±0·15 mg per 100 ml of blood at levels below 3 mg per 100 ml, increasing to ±0·35 mg per 100 ml of blood at levels above 7 mg per 100 ml, a 1 in 10 dilution being used during protein precipitation. At this dilution, the range of the method for each fraction was 0 to 15 mg per 100 ml of blood. The necessity for higher dilution of severely hyperketonaemic blood during protein precipitation or before distillation clearly does not increase the percentage error. The method was therefore accurate at low blood ketone levels, the percentage error being little higher than at moderate or high levels.

Miss Bridget E. Burnett and Messrs. S. H. Buttery, D. R. Williams and S. C. Mills assisted at various stages in the development of the method. Argon Neon Instruments, Surry Hills, Sydney, Australia, assisted with the design of the apparatus.

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The Removal of Interfering Ions in the Determination of Betaine in Sugar-beet Juices and Plant Material

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Betaines are shown not to be adsorbed by an intimate mixture of the hydroxide form of a quaternary amine anion-exchange resin and the hydrogen form of a carboxylic cation-exchange resin. Choline, dimethylglycine, methylglycine, amino acids and ionic substances are quantitatively adsorbed on the resin mixture. A method is described for treating sugar-beet juices or plant extracts with the resin mixture to remove interfering ions, and betaine can be determined in the treated liquor by any quantitative technique, such as precipitation by ammonium reineckate.

In the absence of interfering ions, betaine can be determined by precipitation with potassium tri-iodide,[1,2] phosphotungstic acid[3] or ammonium reineckate. None of these precipitants is specific for betaine, and complex procedures have been described[1] for removing some of the compounds that may be co-precipitated with betaine by potassium tri-iodide.

Walker and Erlandsen[4] used ammonium reineckate at pH 1 to precipitate betaine reineckate and overcame interference caused by precipitation of choline and strong nitrogenous bases by decomposing the crude reineckate with silver nitrate and titrating the acidic betaine nitrate against sodium hydroxide to pH 6. The nitrates of the stronger bases were not sufficiently acidic to be titrated at this pH. The procedure was used without pretreatment for determining betaine in sugar-beet molasses, but it was necessary to clarify beet-diffusion juice with lime before analysis. In applying this method to purified extracts of tissues of young roots and seedlings, Cromwell and Rennie[5] found that other basic substances were co-precipitated with betaine at pH 1 and that the reineckates of some of these substances produced acidic salts when treated with silver nitrate, thus being included in the determination as betaine.

Cromwell and Rennie therefore treated the crude precipitate with silver nitrate to remove reineckate ion and then boiled the solution in the presence of silver oxide to decompose most of the interfering substances. After acidification with hydrochloric acid and separation of the precipitated silver chloride, the betaine was again precipitated by ammonium reineckate and determined by a method similar to that described by Walker and Erlandsen.

In the absence of a complete analysis of plant material, the efficiency of specific chemical treatments for the removal of interfering ions cannot readily be forecast and the procedures are necessarily tedious, so that a single method for separating betaine free from other ionic species was desirable.

Betaine is adsorbed on strongly acidic cation-exchange resins,[6] but no satisfactory procedure is available to elute betaine quantitatively without also eluting amino acids. During studies of combinations of ion-exchange resins for the de-ionisation of beet extracts and process liquors, it was observed that, although almost complete de-ionisation was obtained with mixtures of sulphonic acid cation-exchange and quaternary amine anion-exchange resins, the effluent from the column contained betaine if a weakly acidic carboxylic cation-exchange resin was substituted for the sulphonic acid resin. The latter combination was therefore investigated as a possible method for removing interfering ions before the determination of betaine.

De-ionisation is more efficient with a mixture of acidic and basic resins than with separate columns, but it was found that betaine passed quantitatively through an intimate mixture of the hydroxide form of a strongly basic anion-exchange resin and the hydrogen form of a weakly acidic cation-exchange resin, *e.g.*, De-Acidite FF(OH) *plus* Amberlite IRC-50(H). The resin treatment quantitatively removed all ionic species, other than betaine, known to be present in beet liquors. Since betaine is not adsorbed on the resins, the process is not subject to the disadvantages of other ion-exchange methods, which require recovery of material from the ion-exchange column before analysis.

After removal of interfering ions by passing the solution for analysis through the resin mixture, betaine was determined in the treated liquor by Walker and Erlandsen's[4] reineckate

titration method. For beet molasses and all clarified beet process liquors, the betaine nitrogen, as determined by the above-mentioned method, is equal to the total nitrogen present in the effluent; the betaine would consequently be determined by any quantitative precipitation technique, either colorimetrically, as betaine reineckate, or as nitrogen by the Kjeldahl method.

The effluent from beet extract or unclarified raw juice contains small amounts of nitrogen in excess of the betaine nitrogen; this is probably due to the passage of traces of protein through the resin column. The excess of nitrogen in the effluent has been shown not to interfere with the reineckate - silver nitrate titrations, which, in conjunction with ion-exchange pre-treatment, can be used for unclarified biological materials.

Method

Materials and reagents—

Ion-exchange resin mixture—Convert 500 ml of De-Acidite FF (16 to 50 mesh) to the hydroxide form and 250 ml of Amberlite IRC-50 (16 to 50 mesh) to the hydrogen form by treating them, respectively, with two bed-volumes of 2 *N* sodium hydroxide and ten bed-volumes of *N* hydrochloric acid. Wash the resins free from alkali or acid, drain, and intimately mix two volumes of De-Acidite FF with one volume of Amberlite IRC-50.

Ammonium reineckate solution—Suspend 10 g of ammonium reineckate in 200 ml of water, shake for 30 minutes, filter, and adjust to pH 1 with hydrochloric acid (3 to 4 ml).

Preparation of columns—

Mix 10 ml of the resin mixture into a slurry with just sufficient water to cover the surface of the column of resin in a chromatographic tube prepared by joining a short length of 0·6-mm external diameter glass tubing to the base of a 16-mm × 150-mm test-tube. Avoid the use of an excessive amount of water in transferring the resin mixture to the tube, otherwise partial separation of the resins will occur, as the density of the cation-exchange resin is greater than that of the anion-exchange resin.

Procedure for sugar-beet process juices—

The concentration of betaine in the test solution to be placed on the column should be between 1·5 and 7·5 mg per ml. Suitable amounts are contained in raw juice, thin juice, process syrups diluted to a sucrose content of about 15 per cent. and in 5 per cent. solutions of molasses.

Transfer 10 ml of test solution to the column, and collect the effluent at the rate of about 1 ml per minute. When the liquid level has fallen to the top of the resin, wash the column with water until a total of 50 ml of effluent and washings is obtained.

Acidify a 10-ml aliquot of the combined effluent and washings with 1·0 ml of *N* hydrochloric acid, and add 10 ml of ammonium reineckate solution. Cool to 5° C, and separate the precipitated betaine reineckate on a sintered-glass crucible (porosity No. 3). Wash the precipitate free from acid with 5-ml portions of diethyl ether. Ensure that the crucible is completely free from acid by testing with moist indicator paper, since any residual acid will be determined as betaine; approximately 30 ml of ether are required for complete washing. Dissolve the precipitate in 10 ml of a 70 per cent. aqueous solution of acetone, and finally wash the crucible with 10 ml of water.

To determine betaine in the aqueous acetone solution by Walker and Erlandsen's titration method,[4] add 10 ml of a solution 0·1 *N* in both silver nitrate and sodium nitrate, stir, and filter the mixture through a small Buchner funnel. Wash the precipitated silver reineckate with 20 ml of water, and titrate the betaine nitrate in the combined filtrate and washings with 0·01 *N* sodium hydroxide to the methyl red end-point.

Procedure for plant material—

Place 50 g of chopped root or leaf and 200 ml of water in a 500-ml beaker, weigh the beaker, and boil the contents gently for 5 minutes. Restore the evaporation loss, calculated by weighing, macerate the mixture for 10 minutes in a blender, and filter. (The boiling is merely to facilitate maceration and can be omitted for chopped sugar-beet root. If beet brei is available, a cold-water extract can be prepared with a brei to water ratio of 1 to 4 and only sufficient material to yield 50 ml of filtrate.)

Place 50 to 70 ml of the plant extract on the column, and collect a total of 100 ml of effluent and washings. Evaporate a 50-ml aliquot of the combined effluent and washings

to approximately 10 ml, acidify, and determine betaine by reineckate precipitation as described above. It is desirable to precipitate 3 to 15 mg of betaine; if necessary, the amount of extract placed on the column may be increased, provided that the absorption capacity, approximately 2 millimoles of ionic material, is not exceeded and that the column is washed with not less than 30 ml of water.

RE-USE OF RESINS—

When large numbers of determinations are involved it is possible, because of the greater density of the cation-exchange resin, to separate the two resins for regeneration by backwashing in a column not less than 2 inches in diameter. This separation is not practicable with less than 1 litre of resin, and it is probably more convenient when making infrequent determinations to discard the resin mixture after use.

Amberlite IRC-50 can be obtained in the hydrogen form, so that the acid treatment of this resin is not necessary if the resin is not re-used.

RESULTS

The betaine content of a standard solution of betaine hydrochloride was checked by titration with sodium hydroxide to pH 6. Various aliquots of the solution were diluted to 10 ml and placed on a column of the mixed ion-exchange resins. The betaine contents of the 50-ml portions of effluents were determined by reineckate - silver nitrate titration; the results were—

Betaine in test solution, mg ..	13·8	13·8	27·6	27·6
Betaine found in effluent, mg ..	14·1	13·9	27·9	28·5
Recovery, %	102·2	100·7	100·1	103·3

These recoveries are of the same order as those from standard solutions without column treatment, *i.e.*, betaine is not adsorbed on the resin mixture.

Beet second-carbonatation juice (10 ml) was placed on the column. This juice contains at least twenty common amino acids, principally glutamine, glutamic acid, aspartic acid, alanine, γ-aminobutyric acid, leucine and valine. The effluent was neutral, free from potassium and sodium and formed no colour under the conditions of Moore and Stein's ninhydrin reaction, which is sensitive to 0·5 p.p.m. of α-amino nitrogen.

Choline, methylglycine and dimethylglycine were also completely adsorbed. Trigonelline (N-methyl nicotinic acid betaine) was not adsorbed and was determined quantitatively by the reineckate procedure. It was concluded that the column treatment effectively removed all potential interfering ions except betaines. No betaines other than trimethylglycine have been reported in sugar-beet juices or extracts.

The recovery of standard betaine hydrochloride solution added to beet raw juice before ion-exchange treatment is shown in Table I.

TABLE I

RECOVERY OF ADDED BETAINE FROM BEET RAW JUICE

Sample No.	1	2
Betaine in raw juice, mg per 100 ml ..	143	145
Betaine added, mg per 100 ml ..	139	69·5
Betaine found, mg per 100 ml ..	285	215
Added betaine recovered, mg per 100 ml ..	142	70
Recovery, %	102	101

The betaine and total-nitrogen contents of the 50-ml effluents from a series of 10-ml samples of sugar-beet factory juices, including raw juice, second-carbonatation juice, thick juice diluted to a sucrose content of 15 per cent. and a 5 per cent. solution of molasses, are shown in Table II.

TABLE II

BETAINE AND TOTAL-NITROGEN CONTENTS OF EFFLUENTS FROM BEET PROCESS JUICES

Sample	Betaine-N found, mg	Total nitrogen found, mg
Raw juice	1·40	1·60
	1·46	1·72
Second-carbonatation juice	1·90	1·88
Thick juice	1·95	1·93
Molasses	1·54	1·56

With the exceptions of those of the effluents from raw juice, the betaine and total-nitrogen contents do not differ by more than the probable error of the determinations.

The effluents from raw juice, however, contained nitrogen in excess of the betaine nitrogen. The fact that this excess of nitrogen does not interfere with the determination of betaine is shown by the results in Table III for the analysis of raw juices and the corresponding second-carbonatation juices from different factories.

TABLE III

BETAINE CONCENTRATIONS FOUND IN BEET PROCESS JUICES AND MOLASSES

Factory	Concentration of betaine in— raw juice, parts per 100 parts of sucrose	second-carbonatation juice, parts per 100 parts of sucrose	molasses, parts per 100 parts of sucrose
A	1·25	1·25	4·42
B	1·20	1·22	3·93
C	1·28	1·29	4·35
D	1·18	1·21	3·98
E	1·44	1·41	4·32
F	1·28	1·30	4·00
G	1·27	1·31	3·96
H	1·30	1·27	3·39
Mean	1·28	1·28	4·04

In the production of second-carbonatation juice from raw juice, the protein and about 30 per cent. of the non-sugars are removed, but there are no significant differences in betaine contents, relative to sucrose, of raw juice and second-carbonatation juice. It is therefore unnecessary to clarify raw juice before the ion-exchange treatment.

The concentrations of betaine in samples of molasses are also shown in Table III. Since betaine is not removed in the factory processes it is possible to estimate the molasses production, relative to the sucrose intake, from the relative concentrations of betaine in raw juice and molasses.

Concentrations of betaine in molasses, as determined by Walker and Erlandsen's method[4] without treatment with the resin mixture, were from 9 to 14 per cent. higher than those found by the proposed procedure; this implies that potentially interfering ions are present in molasses.

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A Differential Method of Photometric Analysis

Part II.* Application to Solutions Containing More Than One Component

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The differential method previously applied to one-component systems has been extended to systems containing two and three components.

The application of a differential photometric method to solutions containing single metals[1] gave results similar in accuracy and reproducibility to those obtained by Bastian and others. The method has subsequently been extended to two- and three-component systems containing the perchlorates of nickel, copper, cobalt and chromium in the approximate proportions found in various industrially useful alloys. The experimental procedure used was described in Part I of this series.[1]

Experimental

Effect of interference—

When the calibration curve for a single absorbing species is prepared,[1] the extreme concentrations of the standard solutions are set to give zero and 100 per cent. transmission. The curve is not linear, and its shape at a particular wavelength depends only on the extreme concentrations; hence it is valid at that wavelength for any other absorbing species whose extreme concentrations have the same absolute transmissions as those of the standard. The calibration curve can therefore be used to calculate the effect of interference from a second species, provided that the molecular extinction coefficients of the two species are known. This calculation has been described in standard text-books[2,3] and is similarly applicable to systems containing three components when three wavelengths are used.

For maximum accuracy the wavelengths chosen should be those at which one component absorbs strongly and the others weakly, all the curves being relatively flat.

Solutions used—

The concentrations of the reference and test solutions were determined as described previously.[1] The extreme concentrations of the standard solutions used at each wavelength are shown in Table I, together with the calculated scale expansions obtained by using the differential method.[1]

Table I

Calculated scale expansions for reference solutions

Metal present	Wavelength used, mμ	Extreme concentrations of reference solutions, g per litre	Calculated scale expansion
Nickel	393	2·4000 and 2·7568	15
	720	1·3723 and 3·4550	1·6
Cobalt	510	0·7835 and 1·0970	8
		3·1340 and 5·0144	13·5
Copper	815	0·7900 and 1·1850	10
		0·0 and 1·1850	1·1
Chromium	410, 580	0·9459 and 2·2070	20, 18
		0·0 and 1·2610	1·3, 1·1

Table II shows the concentrations of the test solutions used in the investigation of two- and three-component systems and also the percentage compositions to which these values correspond when regarded as derived from industrial alloys.

* For details of Part I of this series, see reference list, p. 278.

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TABLE II

CONCENTRATIONS OF TEST SOLUTIONS

System No.	Metals present	Concentrations of solutions, g per litre	Corresponding contents of alloy, %
Two-component systems—			
1	Nickel	2·4115	71·95
	Cobalt	0·9402	28·05
2	Nickel	2·4115	71·10
	Copper	0·9875	28·90
3	Nickel	2·4115	60·46
	Chromium	1·5770	39·54
4	Copper	0·1975	23·86
	Chromium	0·6306	76·14
Three-component systems—			
5	Nickel	2·4115	55·58
	Cobalt	0·9402	21·68
	Copper	0·9875	22·76
6	Nickel	2·4115	74·45
	Copper	0·1975	6·08
	Chromium	0·6306	19·47
7	Nickel	2·4115	41·07
	Cobalt	1·8804	32·05
	Chromium	1·5770	26·88

RESULTS

The results in Table III are the means of five sets of concentration determinations carried out at various times by one observer, the same instrument (a Unicam SP500 spectrophotometer) and the same cells being used throughout. Each set of results is the mean of a duplicate determination performed without removing the cells from the carrier. The mean error of the resulting determination is also shown, together with the coefficient of variation of the five results used.

TABLE III

METAL CONTENTS FOUND IN TEST SYSTEMS BY THE PROPOSED METHOD

System No.	Metals present	Metal content found, %	Mean error, %	Coefficient of variation
Two-component systems—				
1	Nickel	72·16	+0·30	0·25
	Cobalt	28·07	+0·07	0·21
2	Nickel	70·90	−0·29	0·10
	Copper	28·79	−0·39	0·17
3	Nickel	60·67	+0·35	0·07
	Chromium	39·63	+0·23	0·10
4	Copper	23·93	+0·28	0·11
	Chromium	76·39	+0·33	0·06
Three-component systems—				
5	Nickel	55·68	+0·19	0·17
	Cobalt	21·63	−0·23	0·14
	Copper	22·71	−0·24	0·18
6	Nickel	74·56	+0·15	0·04
	Copper	6·095	+0·24	0·06
	Chromium	19·49	+0·10	0·08
7	Nickel	41·02	−0·12	0·08
	Cobalt	31·98	−0·21	0·04
	Chromium	26·97	+0·33	0·07

DISCUSSION OF RESULTS

Little work has been done on the analysis of multi-component systems by differential methods. Banks, Spooner and O'Laughlin[4] have applied Reilley and Crawford's method[5] to the analysis of neodymium - yttrium mixtures, the elements being present as perchlorates;

their results at 575 mμ for neodymium were similar in accuracy to those in Table III. Bastian's method has been applied to the analysis of uranium - niobium mixtures by Banks, Burke, O'Laughlin and Thompson,[6] but their results cannot be used for comparison, as the true compositions of the mixtures analysed were not quoted. Bacon and Milner[7] have analysed uranium - molybdenum mixtures and quote results for the minor component, molybdenum; their results are also similar in accuracy to those in Table III.

Conclusions

From the results found, it appears that Reilley and Crawford's method is capable of an accuracy to within ±0·2 per cent. for multi-component systems. It is difficult to institute any valid comparison with existing results for differential methods applied to such systems, but even at the comparatively low scale expansions used the results are equally good. A limiting factor in the accuracy of determinations by this method is the accuracy with which interference can be calculated, which is poor for weakly absorbing materials. Against this, however, the method is the most versatile and potentially the most accurate photometric method in the absence of gross interference.

The experimental work described in this paper contains part of a thesis submitted by one of us (S.D.R.) for the Ph.D. degree of the University of London. The same author expresses his thanks to the Central Research Fund of the University of London for a grant for the purchase of silica absorption cells used in this work.

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Note—Reference 1 constitutes Part I of this series.

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The Absorptiometric Determination of Phenol

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A colorimetric method for determining phenol has been developed; it is based on the reaction between phenol and 4-aminoantipyrine in presence of an oxidant and a buffer solution having a pH between 9 and 10·5. Its use permits rapid determinations in aqueous solutions containing from 2 to 20 p.p.m. of phenol, with an accuracy to within ±5 per cent.

The method described in this paper was devised for use in investigating the bacterial destruction of phenol, when many samples had to be dealt with by one operator in the shortest possible time. For our purpose the existing colorimetric methods had several drawbacks.

Gibbs's method,[1] although sensitive, is laborious; the reagent is not stable, the pH has to be controlled within a narrow range, and substances often encountered in bacterial media interfere and must be removed. Methods based on coupling with diazotised sulphanilic acid[2,3,4] or *p*-nitroaniline[5,6] also suffer from instability of the reagents. The determination of phenol with Millon's reagent[7] has the same drawbacks. The preparation of Folin and Denis's reagent[8] is not easy, and interfering substances, such as sugar, proteins, etc., must be removed.

The proposed method is based on the reaction of 4-aminoantipyrine[9] in presence of an oxidant with phenols having the *para*-position either free or occupied by a halogen, carboxyl, sulphonic acid, hydroxyl or methoxyl group. The product of the reaction is a red compound.

At a pH of 8 or less, 4-aminoantipyrine is itself oxidised to a red compound; however, at pH values above 8 the colour of this compound changes to yellow, but that of the phenol - 4-aminoantipyrine compound remains red. In order to create suitable conditions, *i.e.*, an oxidising medium and pH about 10, potassium ferricyanide and various amounts of ammonia or sodium carbonate have been used,[10,11,12,13] the product of the reaction then being extracted into chloroform. This procedure was too lengthy and laborious for our purpose, but, when the extraction was omitted, results were not reproducible and the colour sometimes faded rapidly. This fading was prevented by using ammonium or potassium persulphate instead of potassium ferricyanide, and its cause was later traced to a reaction between 4-aminoantipyrine, carbonate and ferricyanide. When more 4-aminoantipyrine and oxidant were added to a faded solution the red colour was re-formed. This could be explained as follows. In the aqueous mixture of 4-aminoantipyrine, phenol, ferricyanide and carbonate, two main reactions take place, *viz.*, the reaction between 4-aminoantipyrine and phenol and the oxidation of 4-aminoantipyrine. In presence of carbonate, phenol is split off from the product of the first reaction and the free 4-aminoantipyrine is then oxidised. When more 4-aminoantipyrine and oxidant are added, the liberated phenol reacts again.

The results were unaffected either by the nature of the buffer solution, when its pH value was between 9 and 10·5, or by changes in temperature between 19° and 40° C. 4-Aminoantipyrine is oxidised to an acid, and a buffer solution was therefore added to stabilise the pH. A saturated solution of sodium tetraborate (pH 10·1) was chosen in preference to other buffer solutions because of the simplicity of its preparation. The maximum light absorption of the reaction mixture was at 500 mμ when a phosphate or carbonate buffer solution was used and at 540 mμ with the sodium tetraborate solution. Calcium, magnesium and iron salts encountered in water do not interfere, neither do amino acids or peptides.

METHOD

REAGENTS—

4-Aminoantipyrine solution—Dissolve 3 g of technical-grade 4-aminoantipyrine in 100 ml of distilled water, filter the solution if it is cloudy, and store in a cool dark place. This solution is stable for about 2 weeks.

Ammonium persulphate solution—Dissolve 2 g of analytical-reagent grade ammonium persulphate in 100 ml of distilled water. Store the solution in a cool dark place; it is stable for at least 2 weeks.

Sodium tetraborate solution—Dissolve 40 g of sodium tetraborate in 1 litre of hot distilled water; set aside overnight to cool and to allow excess of solute to crystallise. Use the supernatant solution.

Standard phenol solutions—Accurately weigh about 1 g of pure phenol, dissolve in 100 ml of the sodium tetraborate solution, transfer to a 1-litre calibrated flask, and dilute to the mark with distilled water. Store in a cool dark place. By suitable dilution of this solution, prepare separate solutions to cover the range 0·5 to 20 p.p.m. of phenol. These solutions are not stable and must be used immediately after preparation.

PREPARATION OF CALIBRATION GRAPH—

Place 5 ml of standard phenol solution in a 50-ml calibrated flask, add 10 ml of sodium tetraborate solution, and mix well. Add 1 ml of 4-aminoantipyrine solution, mix again, wash down the neck of the flask with a further 10 ml of sodium tetraborate solution, and then add 1 ml of ammonium persulphate solution. Shake well, and set aside for 10 minutes to allow colour development. Dilute to the mark, and measure the light-absorption difference between the standard solution and a blank solution containing no phenol; use a Spekker absorptiometer, 4-cm cells and Ilford No. 604 filters. A graph of the results against the concentrations of phenol present should be linear and pass through the origin.

PROCEDURE—

If the sample is cloudy, filter or spin in a centrifuge. Treat the clear solution as described above, and determine the concentration of phenol present in the sample by reference to the calibration graph.

DISCUSSION OF THE METHOD

The results quoted in this section were obtained by the proposed procedure, all light-absorption measurements being made against a blank solution.

EFFECT OF SUBSTANCES PRESENT IN MEDIA—

Solutions of phenol were prepared in tap-water containing about 100 p.p.m. of calcium, 5 p.p.m. of magnesium, traces of iron and 100 p.p.m. of Difco yeast extract. The results in Table I show that the presence of these substances had no appreciable effect on the recovery of phenol.

TABLE I

RECOVERY OF PHENOL ADDED TO TAP-WATER CONTAINING CALCIUM, MAGNESIUM, IRON AND YEAST EXTRACT

Phenol added, p.p.m.	Phenol found, p.p.m.	Recovery, %	Phenol added, p.p.m.	Phenol found, p.p.m.	Recovery, %
1·03	1·0	97	12·38	12·2	98·5
2·06	2·0	97	14·42	14·4	99·8
4·12	3·9	95	16·5	16·3	98·8
6·18	6·2	100·2	18·55	17·9	96·5
8·24	8·2	99·5	20·6	19·6	96·6
10·30	10·3	100			

TABLE II

LIGHT-ABSORPTION VALUES OF PHENOL SOLUTIONS IN PRESENCE OF BORATE, PHOSPHATE AND CARBONATE BUFFER SOLUTIONS

	Light-absorption value of solution containing—					
pH	2 p.p.m. of phenol, absorptiometer-drum units	4 p.p.m. of phenol, absorptiometer-drum units	8 p.p.m. of phenol, absorptiometer-drum units	12 p.p.m. of phenol, absorptiometer-drum units	16 p.p.m. of phenol, absorptiometer-drum units	20 p.p.m. of phenol, absorptiometer-drum units
In presence of borate buffer solution—						
9·0	0·12	0·23	0·46	0·67	0·82	1·10
9·6	0·12	0·23	0·45	0·67	0·88	1·09
10·0	0·11	0·22	0·45	0·67	0·87	1·08
10·6	0·12	0·23	0·45	0·66	0·87	1·05
In presence of phosphate buffer solution—						
7·7	0·08	0·22	0·41	0·65	0·84	1·04
9·2	0·13	0·24	0·45	0·65	0·87	1·05
10·7	0·13	0·25	0·44	0·64	0·84	1·03
11·0	0·10	0·20	0·38	0·54	0·72	0·89
11·5	0·08	0·15	0·31	0·44	0·56	0·70
In presence of carbonate buffer solution—						
10·5	0·11	0·21	0·62	0·62	0·82	1·01

TABLE III

EFFECT OF NORMAL VARIATION IN TEMPERATURE

	Light-absorption value at—			
Phenol present, p.p.m.	19° C, absorptiometer-drum units	22° C, absorptiometer-drum units	25° C, absorptiometer-drum units	37° C, absorptiometer-drum units
2	0·11	0·12	0·11	0·10
4	0·23	0·23	0·23	0·23
8	0·44	0·44	0·44	0·44
12	0·85	0·85	0·85	0·84
20	1·04	1·05	1·05	1·04
24	1·30	1·30	1·29	1·29

EFFECT OF pH OF BUFFER SOLUTIONS—

Borate, phosphate and carbonate buffer solutions were used in investigating the effect of pH on colour formation. Borate and phosphate buffer solutions were prepared by mixing

various proportions of 0·1 *M* solutions of sodium hydroxide and boric acid or potassium dihydrogen orthophosphate; the carbonate buffer solution was 0·1 *M* sodium sesquicarbonate. The results in Table II indicate that maximum colour formation for a given concentration of phenol occurs at a pH between 9 and 10·7 and that the chemical composition of the buffer solution has no effect on the light absorption.

EFFECT OF TEMPERATURE—

Normal variations in laboratory temperature had no effect on the light-absorption values (see Table III). However, when the light absorption of a solution containing about 18 p.p.m. of phenol was measured at various temperatures, the colour was found not to be stable above 50° C; the results were—

Temperature, °C	25	30	40	50	60	70	80	90
Light-absorption value, absorptiometer-drum units	0·94	0·94	0·94	0·94	0·93	0·78	0·50	0·28

EFFECT OF PURITY OF 4-AMINOANTIPYRINE—

Various batches of commercial 4-aminoantipyrine were used in the determinations. Some formed dark orange solutions and had a melting-point as low as 103° C, whereas pure 4-aminoantipyrine, melting-point 109° C, formed pale yellow solutions. A series of light-absorption measurements was made in which two batches of 4-aminoantipyrine were used; batch A melted at 103° C and batch B at 109° C. It was found that the degree of purity of the reagent had no effect on the measurements; the results were—

Phenol present, p.p.m.	2·06	4·12	8·24	12·36	16·5	20·6
Light-absorption value when batch A was used, absorptiometer-drum units	0·12	0·23	0·45	0·65	0·85	1·05
Light-absorption value when batch B was used, aborptiometer-drum units	0·12	0·23	0·45	0·65	0·86	1·05

Old batches of 4-aminoantipyrine could be satisfactorily purified by washing with small amounts of benzene.

STABILITY OF COLOUR—

The results shown below indicate that colour was fully developed within 5 minutes and was unchanged after 150 minutes.

Phenol present, p.p.m.	2·0	4·1	8·2	12·3	16·5	20·6
Light-absorption value after 5 minutes, absorptiometer-drum units	0·11	0·22	0·45	0·67	0·86	1·08
Light-absorption value after 150 minutes, absorptiometer-drum units	0·10	0·21	0·44	0·64	0·85	1·08

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The Rapid Radiochemical Determination of Caesium-137

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A method is described for the rapid radiochemical determination of caesium-137 in various materials containing 0·1 g or less of potassium. The first step is scavenging with ferric hydroxide (or phosphate), the second is precipitation of caesium with potassium dipicrylaminate and the third is re-precipitation of caesium as chloroplatinate. The beta-particle activity of the final precipitate (4 to 15 disintegrations per minute) is counted by a low-level counting system. The combined contribution of the activities of potassium-40 and rubidium-87 is less than 0·2 disintegration per minute, and decontamination factors for other fission activities are greater than 10^4. The method can be applied to the determination of caesium-137 in 50-g wet samples of human tissue or in 10-g samples of bone.

During 1957 and 1958 various foodstuffs, human urine and blood in Japan were found to contain from 20 to 70 $\mu\mu$c of caesium-137 per g of potassium.[1] By using a low-background beta-counting system, 2 to 7 $\mu\mu$c of caesium-137 can be conveniently determined with an error of less than 10 per cent. This amount of caesium-137 is associated with approximately 0·1 g of potassium in natural materials. We studied the separation of caesium-137 from a large amount of potassium and other elements and recommended the combined molybdophosphate - chloroplatinate method for samples containing 0·2 to 0·5 g of potassium.[2] In presence of less potassium, however, caesium-137 can be conveniently co-precipitated with potassium dipicrylaminate. In this paper a combined dipicrylaminate - chloroplatinate method for determining caesium-137 is described.

The method is intended for samples containing 0·1 g or less of potassium and is based on three separations. The first is the scavenging of other fission activities by co-precipitation with ferric hydroxide, the second is co-precipitation of caesium with potassium dipicrylaminate and the third is re-precipitation of caesium as chloroplatinate.

Method

Reagents—

Caesium carrier solution, 10 mg per ml—Dissolve 1·267 g of caesium chloride in water, and dilute to 100 ml.

Sodium hydroxide solution, 2 per cent.

Thymol blue indicator solution—Mix 100 mg of thymol blue with 2·15 ml of 0·1 *N* sodium hydroxide, and dilute to 100 ml with water.

Sodium dipicrylaminate solution—Mechanically agitate 25 g of dipicrylamine with 500 ml of water, and add sodium hydroxide until solution is almost complete. Set aside for several hours, and filter.

iso*Butyl methyl ketone.*

Hydrochloric acid, 2 N.

Ethanol.

Diethyl ether.

Chloroplatinic acid solution—A 10 per cent. w/v solution of chloroplatinic acid, $H_2PtCl_6.6H_2O$, in water.

Procedure—

Subject the sample containing approximately 0·1 g of potassium (see Table I) to wet oxidation or dry ashing. (Note that samples of radioactive fall-out, soil extracts or water equivalent to between 2 and 7 $\mu\mu$c of caesium-137 may contain less than 0·1 g of potassium; to such samples add a known amount, approximately 0·2 g, of pure potassium chloride.) If dry ashing is used and organic matter remains, treat the residue with several millilitres of nitric - hydrochloric acid mixture (1 + 3), and evaporate to dryness. Repeat this procedure until the organic matter has been destroyed.

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TABLE I

APPROXIMATE AMOUNTS OF SAMPLES EQUIVALENT TO 0·1 g OF POTASSIUM

0·1 g of potassium is associated with 2 to 7 $\mu\mu$c of caesium-137

Sample	Amount required	Sample	Amount required
Dried milk	20 g	Raw vegetables	50 g
Raw cereal	100 g	Raw meat	50 g
Urine (human)	100 ml	Raw tissue (human) ..	50 g
Raw bone (human) ..	10 g	Blood (human)	100 ml

To between 100 and 200 ml of an acid solution of the ash add 20 mg of iron, as ferric chloride solution, and 2 ml of caesium carrier solution. Heat almost to boiling-point, and then add 2 per cent. sodium hydroxide solution until a precipitate of ferric hydroxide appears. Add a few drops of thymol blue indicator solution, and continue to add sodium hydroxide solution until the colour changes from yellow through green to blue. Set aside for a short period, separate the precipitate on a filter-paper of loose texture, wash once with hot water, and add the washings to the filtrate. If necessary, adjust the pH of the combined filtrate and washings to between 8 and 9 by adding dilute hydrochloric acid or sodium hydroxide solution.

Cool the solution in ice - water mixture, add about a 100 per cent. excess of cooled sodium dipicrylaminate solution, with constant stirring, and continue to stir for 15 minutes. Set aside for not less than 30 minutes at between 0° and 5° C, filter through a weighed sintered-glass crucible, and wash the precipitate with 10 ml of ice-cold water and then 10 ml of diethyl ether at between 0° and 5° C. Dry the crucible and precipitate at 105° C for 30 minutes, allow to cool, and weigh (weight of precipitate = x g).

Dissolve the dry precipitate in about 20 ml of *iso*butyl methyl ketone, place the solution in a separating funnel, and add 20 ml of 2 *N* hydrochloric acid. Shake the funnel vigorously for 1 minute, run the aqueous layer into a 200-ml beaker, and repeat the extraction with two further 20-ml portions of 2 *N* hydrochloric acid (see Note). Combine the aqueous layers, evaporate to dryness, and dissolve the residue in 100 ml of distilled water. To this solution add 1·5 ml of chloroplatinic acid solution, with stirring, rub the wall of the beaker with a glass rod until a yellow precipitate appears, and set aside for several hours. Separate the precipitate on a weighed 1-inch filter-paper supported on a Hirsch funnel, and wash with 10 ml of cold water and then 10 ml of ethanol. Dry at 110° C, cool, and weigh to determine the recovery of caesium (weight of precipitate = y g).

Mount the dried filter-paper and precipitate on a stainless-steel counting tray, count with a low-background beta counter, and correct the observed activity for self-absorption and scattering of caesium chloroplatinate by reference to a previously prepared calibration graph. The calibration graph is prepared by plotting the activities of standard samples of caesium-137 (obtainable from the National Bureau of Standards, Washington, U.S.A.) precipitated, as chloroplatinate, together with different amounts of added caesium carrier.

NOTE—Free dipicrylamine is less soluble in *iso*butyl methyl ketone than is the potassium salt. As a result, a yellow precipitate may appear and render separation of the layers incomplete. If this happens, pass both layers through a filter-paper moistened with water; this removes the precipitate (free dipicrylamine *plus* drops of *iso*butyl methyl ketone).

CALCULATION—

Calculate the activity of the original sample from the expression—

$$\text{Activity } (E),\ \mu\mu\text{c} = \frac{\text{Corrected activity of final precipitate, } \mu\mu\text{c} \times 0{\cdot}0507}{y},$$

and deduce the amount of caesium-137 present per g of potassium in the original sample (the caesium unit) from the expression—

$$\text{Caesium present, } \mu\mu\text{c} = \frac{E}{(x - 0{\cdot}0859) \times 0{\cdot}0819}.$$

DISCUSSION OF THE METHOD

The effective scavenging of fission activities other than that of caesium with ferric hydroxide (or phosphate) has been reported by many workers; we have shown that more than 99 per cent. of cerium and yttrium can be removed by only 16·8 per cent. of strontium,[2] the strontium being effectively removed by further separations.

The co-precipitation of caesium with thallous dipicrylaminate[3] produced about a 90 per cent. yield of caesium and contamination of less than 1 per cent. of each of the rare-earth elements, strontium, yttrium, zirconium, niobium, ruthenium and rhodium. It is only natural to suppose that caesium will be successfully co-precipitated with potassium dipicrylaminate also. The chemical and radiochemical yields of potassium and caesium-137 obtained by the proposed method are shown in Fig. 1, which indicates favourable co-precipitation of caesium with potassium dipicrylaminate. The chemical yield of potassium under the proposed conditions was over 98 per cent., and the radiochemical yield of caesium was almost 100 per cent. The effectiveness and simplicity of the solvent-extraction procedure for separating potassium and caesium from dipicrylamine have been previously described in detail.[3]

TABLE II

CONCENTRATIONS OF CAESIUM-137 IN HUMAN TISSUES AND ORGANS

The samples were taken from the body of a man who died on April 25th, 1959, from heart failure at the age of twenty-eight. The weight of the body was 57 kg

Sample	Concentration of potassium, mg per g of fresh sample	Concentration of caesium-137, mg per g of fresh sample	Caesium unit, $\mu\mu$c per g of potassium
Muscle	2·33	0·127	54·5
Skeleton (rib)	0·88	0·328	373
Stomach	1·53	0·192	126
Large intestine ..	0·935	0·0268	28·7
Liver	1·89	0·128	67·7
Brain	2·72	0·209	76·8
Lung	1·50	0·0165	11·0
Kidney	1·56	0·0855	54·8
Heart	2·01	0·0794	39·5
Spleen	1·74	0·0138	7·9
Urinary bladder ..	0·920	0·0855	92·9
Pancreas	1·89	0·100	52·9

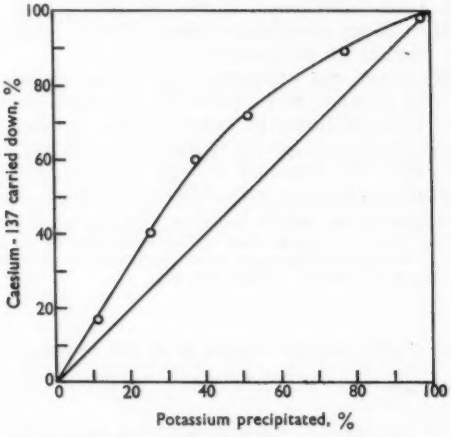

Fig. 1. Graph showing efficiency with which a trace of caesium-137 is carried down by a precipitate of potassium dipicrylaminate

The differences in solubility of the alkali chloroplatinates in water are sufficient for the removal of potassium and rubidium in the last stage of the procedure. The contribution of the activities of potassium-40 and rubidium-87 was computed from the results of flame-photometric analyses of the precipitate, which was found to contain less than 50 μg of potassium and several micrograms of rubidium. As the beta-particle energy of rubidium-87

(0·275 MeV) is considerably less than that of caesium-137 (0·52 MeV), the greater absorption minimises the contribution. The combined contribution of the activities of alkali elements is calculated to be less than 0·2 disintegration per minute.

The over-all decontamination from other fission activities has been examined for strontium, cerium and yttrium by using radioactive tracers, strontium-89, cerium-144 and yttrium-91. The results showed that the decontamination factor is approximately 10^5 for each of the nuclides and greater than 10^4 altogether. This seems to be satisfactory for dealing with environmental contamination by radioactive fall-out.

Various organs and tissues from eighteen autopsied human bodies have so far been analysed by the proposed method. The results showed wide individual variations in the concentration of caesium-137. The details will be published elsewhere, but Table II shows typical results for a man who died of heart failure.

The standard deviation of the determination is approximately 10 per cent. for most samples. The concentration of caesium-137 is highest in the skeleton (rib), both on the fresh basis and the potassium basis (caesium unit). The wide variation in the values of the caesium unit could be explained in terms of the variable ratio of natural caesium to potassium in different organs, but further discussion should not be made until analytical results for natural caesium have been obtained; a neutron-activation method could be used for this determination. The proposed method is thought to be applicable to the separation of caesium-134 after neutron activation.

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Isotopic Determination of Lithium by Neutron Activation

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A method has been developed for determining the isotopic abundance in lithium samples. A solution of lithium hydroxide is irradiated in the neutron flux of a reactor, and the activity of the fluorine-18 produced by the reactions ^{6}Li (n,t) ^{4}He and ^{16}O (t,n) ^{18}F is measured in a positron counter. The error of the method is 1 to 2 per cent., which makes it particularly suitable for the analysis of samples from a small research separation apparatus.

There are already in use several methods of determining the isotopic abundance of lithium; of these the mass-spectrometric method is undoubtedly the most reliable. Several spectrographic methods have been developed, including one by Stukenbroeker *et al.*[1] based on the isotopic spectrum shift of the resonance line at 6707·8 A, which has an error of about 2 per cent. Kaplan and Wilzbach[2] measured the isotopic ratio of lithium by using the (n,α) reaction on lithium-6 and then separated and assayed the tritium formed with an error of 1 per cent., but to obtain sufficient tritium for an accurate measurement the sample had to be irradiated for 2 weeks in a flux of 10^{12} neutrons per sq. cm per second. Other radiometric methods[3,4] have been developed, but these give only approximate results.

In the method described below, the sample, in the form of hydroxide solution, is irradiated with thermal neutrons to produce tritons by the reaction ^{6}Li (n,t) ^{4}He. These tritons then react with the oxygen in the solution to yield fluorine-18, from the ^{16}O (t,n) ^{18}F reaction.

The activity of fluorine-18 (a positron emitter with a half-life of 112 minutes) can be conveniently detected in a positron counter developed by Weale.[5] The counter is sufficiently specific for positron detection to make chemical separation of fluorine-18 unnecessary.

This method has an error of 1 to 2 per cent. and requires about 5 mg of lithium; it is therefore suitable for analysing samples from a small research isotopic-separation apparatus. The method is not an absolute one, as it depends on at least one mass-spectrometer analysis.

Experimental

Positron counter—

A positron on annihilation emits two 0·51-MeV γ-rays in opposite directions; two scintillation counters placed on opposite sides of the emitter recording only events occurring coincidentally in both counters is therefore a specific method of counting positrons.

A positron counter developed by Weale[5] makes use of this principle. It consists of two cylindrical sodium iodide (thallium activated) crystals on opposite sides of the radioactive source. The source is sandwiched between two $\frac{1}{8}$-inch aluminium plates and positioned half-way between the two crystals and along the axis of the system. Each crystal is viewed by a photomultiplier tube, the output from each being amplified and fed into a single-channel pulse analyser adjusted to admit only the 0·51-MeV photopeak. The pulses are then fed into a coincidence unit, and scalers record the coincidences and also the individual channel counts.

Initial experiments—

A 0·1-ml portion of 0·1 *N* natural lithium hydroxide solution was sealed in a silica tube, 2 mm in diameter and 60 mm long, and irradiated in a flux of 10^{12} neutrons per sq. cm per second for 30 minutes. The sample was transferred to the positron counter, and the activity was recorded once every 30 minutes for 6 hours. A decay curve was plotted that corresponded to a pure 112-minute activity.

An empty silica tube irradiated alongside the lithium solution was also counted at intervals of 30 minutes. Again a 112-minute activity was observed, the intensity being about one-third that of the natural lithium solution. Spectroscopic analysis of the silica showed the presence of about 20 p.p.m. of lithium impurity; this was sufficient to account for the observed positron activity. Because of this high blank value, all subsequent lithium solutions were transferred to standard aluminium source holders before counting.

Method

Procedure—

Clean six silica tubes, 80 mm long × 2 mm diameter, closed at one end, with 16 *M* nitric acid and distilled water, and then dry. Rinse the tubes with a 10 per cent. solution of dimethyldichlorosilane in carbon tetrachloride, and again dry.

Place approximately 0·1-ml portions of the lithium solution to be analysed in two of the silica tubes, and seal so that the over-all lengths of the tubes are 60 to 65 mm. Similarly fill two tubes with a second unknown sample and two more with a solution of known lithium-6 content. (It would be advantageous, but not essential, to choose for a standard a solution containing the same order of lithium-6 concentration as the unknown.) Pack the tubes regularly round the sides of an irradiation can, and irradiate for 30 minutes in a flux of 10^{12} neutrons per sq. cm per second.

After irradiation, open the tubes, and transfer each solution to a polythene weight pipette. Transfer an accurately known weight of each solution to a thin plastic film supported by a rectangular plate with a central 1-inch diameter hole, and evaporate to dryness under an infra-red lamp. Determine the activities of the sources in the positron counter (record at least 20,000 counts for each source). Repeat the measurements after about 2 hours as a rough check on the purity of the fluorine-18.

Determine the normality of the lithium hydroxide solution by titrating against *N* hydrochloric acid. (By using a micrometer-syringe pipette to deliver the hydrochloric acid it is possible to titrate a few milligrams of lithium reasonably accurately and so conserve valuable material.)

To calculate the percentage abundance of lithium, correct the activities of the unknown and the standard by extrapolating back to the end of the irradiation. Then, if the standard solution containing *S* gram atoms of lithium-6 per litre has an activity of X_s at the end of irradiation and X_u is the activity of the unknown sample of normality *N*, the abundance of lithium-6 in the unknown sample is—

$$\frac{S\,X_u}{N\,X_s} \times 100 \text{ per cent.}$$

Assessment of the method

Self-absorption of neutrons—

The high cross-section of lithium-6 for thermal neutrons tends to reduce the neutron flux at the centre of the sample. In order to keep the self-absorption to a minimum a dilute lithium solution was irradiated in the form of a narrow cylinder. Solutions having different lithium-6 contents were irradiated and counted; the results are shown in Table I. The results show that, for solutions containing less than 0·1 gram atom of lithium-6 per litre, the self-absorption of neutrons in negligible.

Table I

Irradiation of dilute solutions of lithium

Lithium-6 content (A), gram atoms per litre	Counts per minute (B)	$\frac{B}{A} \times 10^{-6}$
0·09753	73,880	7·575
0·07918	59,500	7·515
0·06084	46,190	7·592
0·05178	38,920	7·516
0·04249	31,960	7·522
0·03360	25,500	7·589
0·02414	17,840	7·390
0·00620	4650	7·500
	Mean	7·525
	Standard deviation	±0·061

Effect of impurities—

Two sources of lithium have been used throughout this investigation, namely, enriched lithium-6 hydroxide and laboratory-reagent grade lithium hydroxide (obtained from the British Drug Houses Ltd.). The fluorine-18 produced on irradiation of both products decayed with a half-life of 122 minutes for at least three half-lives. However, the possible production of other nuclides that interfere with the determination has been considered, and the maximum permissible amounts have been estimated.

The most likely source of interference is from thermal neutron reactions producing positron emitters. Fortunately, only one positron-emitting nuclide, copper-64, is formed from thermal neutron reactions in a yield likely to interfere with the main reaction. Nuclides emitting two γ-rays in cascade with both energies equal to or greater than 0·5 MeV may be counted, but with lower efficiencies than positrons. It seems unlikely that (t,n) reactions on elements other than oxygen will interfere because of the dilute solutions irradiated.

Dilute solutions of the most likely impurities in lithium hydroxide have been irradiated and then counted in the positron counter. The results (see Table II) show that the lithium hydroxide must be extremely impure before activities resulting from these impurities affect the accuracy of the determination of lithium-6.

Table II

Effect of impurities

Impurity	Concentration of impurity causing 1 per cent. error in lithium-6 result,* %
Copper	0·11
Sodium	9·0
Potassium	⩾50
Calcium	⩾50
Manganese	0·15
Chromium	1·1
Iron	9·0

* Calculated for 0·1 N lithium hydroxide (7·5 per cent. of lithium-6).

The accuracy of the method is more likely to be affected by impurities, such as sodium and potassium, in the determination of normality. These can be separately determined by flame photometry, and the results for total lithium corrected if they are present in sufficient quantity.

ACCURACY OF THE METHOD—

The results obtained to show the absence of self-absorption up to 0·1 gram atom of lithium-6 per litre indicate that the error of the counting procedure is less than 1 per cent. However, this is not a fair determination of the accuracy because the solutions were prepared by diluting one strong solution and calculating the lithium-6 concentration. To determine the accuracy of the method the lithium-6 contents of three independent solutions were determined by mass spectrometry and by the proposed procedure; the results are compared in Table III. The error is ±2 per cent. for these solutions of low lithium-6 content. This would improve slightly for solutions containing more lithium-6 because of better counting statistics.

TABLE III

COMPARISON OF RESULTS BY DIFFERENT METHODS

Lithium-6 found by mass spectrometry, atom %	Lithium-6 found by neutron activation, atom %	Difference
23·37	23·37	0·0
4·76	4·64	−0·12
	4·60	−0·16
5·43	5·56	+0·13
	5·39	−0·04
	5·50	+0·07
	5·48	+0·05

CONCLUSIONS

The neutron-activation technique provides an alternative method for the isotopic analysis of lithium. It requires fairly small samples and the accuracy is sufficient for most purposes.

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A Titrimetric Method for Determining Arsenite in Presence of Arsenate

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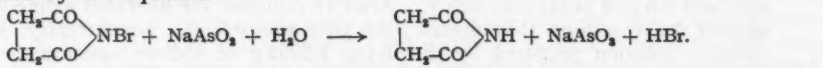
In presence of sodium hydrogen carbonate N-bromosuccinimide readily and quantitatively oxidises aqueous solutions of sodium arsenite to sodium arsenate. The reaction takes place at room temperature, and N-bromosuccinimide is irreversibly reduced to succinimide, with the formation of hydrogen bromide. The determination of sodium arsenite and arsenious oxide by titration with standard N-bromosuccinimide solution is described. The experimental error does not exceed ±2 per cent. Results are reported for comparative analyses of the same samples by the proposed method and by the generally accepted iodine method.

SODIUM arsenite has been used in the treatment of sheep helminthiasis,[1,2] and arsenites have proved to be effective insecticides,[3,4] *e.g.*, sodium arsenite - lime dust for *Anabrus simplex*.[5] This wide use in veterinary medicine has stimulated our interest in the determination of arsenite.

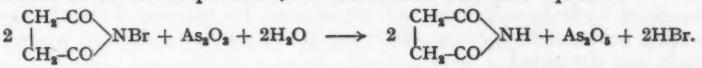
Chemical methods[6,7,8,9] have been extensively applied in the determination of arsenite, the oxidation property of arsenite being used as the basis of the reaction for its determination. The complexity of oxidation - reduction reactions by oxygen-containing oxidising agents has been shown by the anomalous course of the reduction - oxidation potential curve for the titration of arsenite with potassium dichromate solution.[10] Moreover, in potentiometric oxidation - reduction titrations of arsenite with bromate or iodate solution, the titration curves are not always superimposable.[11] In amperometric titrations, standard hypobromite solution is said to be less stable than standard hypochlorite solution in presence of excess of bromide.[12]

The work described here involves the use of N-bromosuccinimide for determining arsenite, even in presence of arsenate. The method is based on the fact that N-bromosuccinimide readily and quantitatively oxidises an aqueous solution of arsenite to arsenate and is itself irreversibly reduced to succinimide, with the formation of hydrogen bromide, as represented by the equation—



In presence of sodium hydrogen carbonate, the reaction is quantitative for equimolecular concentrations at room temperature. Sodium hydrogen carbonate removes hydrogen bromide as it is formed and so helps the reaction to proceed to completion. Sodium carbonate or sodium hydroxide cannot be used, since these compounds react with the bromine to form bromide, hypobromite and bromate. Succinimide has been isolated from the reaction mixture and identified by comparison of the melting-point and mixed-melting-point with that of a pure sample. The presence of arsenate and hydrogen bromide in the reaction solution has been confirmed.

Similarly, N-bromosuccinimide is quantitatively and rapidly reduced to succinimide by arsenious oxide at room temperature, in accordance with the equation—



The addition of sodium hydrogen carbonate also helps this reaction to proceed to completion. In this reaction, one molecule of arsenious oxide is quantitatively oxidised to arsenic oxide, two molecules of N-bromosuccinimide being involved in the reaction.

The reaction between N-bromosuccinimide and arsenite or arsenious oxide does not appear to have been described in the literature. N-Bromosuccinimide is an oxidising agent, often highly selective,[13,14] and can liberate iodine from potassium iodide in aqueous medium, but it oxidises arsenite or arsenious oxide preferentially. Until all the arsenite or arsenious oxide present has been oxidised, no iodine is liberated from potassium iodide. The slightest excess of N-bromosuccinimide added after all the arsenite or arsenious oxide has been oxidised liberates iodine from potassium iodide; this can be easily detected by the blue colour developed with a few drops of starch solution added at the beginning of the titration. Arsenate does not interfere in the titration, and arsenite can consequently be determined in its presence.

N-Bromosuccinimide is analogous to iodine; it acts as an oxidising agent, but is superior because it is a definitely crystalline compound, readily soluble in hot distilled water without volatilisation. An aqueous solution can be easily prepared and keeps well when placed in a dark bottle immediately after preparation. Moreover, N-bromosuccinimide solution does not require standardisation, as does an iodine - potassium iodide aqueous solution, and can be used to determine accurately amounts of arsenious oxide as small as 100 μg (see Table IV). Further, the price of N-bromosuccinimide is more reasonable than are those of iodine and potassium iodide.

Experimental

Reaction between N-bromosuccinimide and arsenite—

A 1·2992-g portion of sodium arsenite (0·01 mole) was dissolved in 30 ml of distilled water, and 1·78 g of N-bromosuccinimide (0·01 mole) were dissolved in 100 ml of hot distilled water. When the N-bromosuccinimide solution was cool, it was gradually added, with shaking, to the cold arsenite solution. The mixture was a colourless solution in which the presence of arsenate[15] was established by means of the tests described below.

Test A—To 10 ml of the colourless solution were added 5 ml of magnesia mixture; a white crystalline precipitate of ammonium magnesium arsenate was formed. When this precipitate was treated with silver nitrate solution containing a few drops of acetic acid, brown silver arsenate was formed.

Test B—To 10 ml of the colourless solution were added 5 ml of nitric acid, and the mixture was heated until all bromine had been removed. Five millilitres each of ammonium molybdate solution and nitric acid were added, and the solution was heated to boiling-point. A yellow crystalline precipitate of ammonium arsenomolybdate was formed.

To confirm the presence of arsenate and to avoid the probability that the nitric acid used in this test would oxidise at least some of the arsenite if any were still present, a further experiment was carried out. To 10 ml of an aqueous solution containing 1·2992 g of sodium arsenite per 100 ml were added 15 ml of concentrated hydrochloric acid and 0·5 g of potassium iodide. After 15 minutes, the liberated iodine was titrated against 0·1 *N* sodium thiosulphate; the titre was 0·5 ml. Concurrently, 10 ml of an arsenate solution prepared by dissolving 1·2992 g of sodium arsenite and 1·78 g of N-bromosuccinimide in 100 ml of distilled water in a calibrated flask were titrated against 0·1 *N* sodium thiosulphate; the titre was 18·8 ml. The blank titration required 0·5 ml of 0·1 *N* sodium thiosulphate, *i.e.*, the same titre as that found when arsenite was titrated.

By quantitative determination it was found that 1·331 g of sodium arsenate were formed from 1·2992 g of sodium arsenite; the theoretical yield is 1·4592 g of sodium arsenate, *i.e.*, 91·21 per cent. of the arsenite present was oxidised to arsenate.

Test C—To 5 ml of the colourless solution were added 5 ml of potassium iodide solution and 2 ml of concentrated hydrochloric acid. When the mixture was shaken with 2 ml of chloroform, the organic layer was coloured violet.

The presence of hydrobromic acid was established by treating 5 ml of the colourless solution with nitric acid and 10 per cent. silver nitrate solution; a yellowish white precipitate of silver bromide was deposited

Succinimide was isolated as follows. The remainder of the colourless solution (100 ml) was distilled off *in vacuo*, and the solid residue was recrystallised from benzene. The colourless crystals so obtained melted at 125° to 126° C and were proved to be succinimide by melting-point and mixed-melting-point determinations with an authentic sample.

VALIDITY OF THE REACTION FOR QUANTITATIVE DETERMINATION—

Before the reaction was applied to the determination of arsenite in test solutions, it was decided to verify the reaction between N-bromosuccinimide and arsenite from a quantitative point of view. An accurately measured volume, *e.g.*, 2 ml, of a solution containing 0·12992 g (1 millimole) of sodium arsenite per 100 ml was placed in a 25-ml conical flask, and an equal volume of 10 per cent. w/v sodium hydrogen carbonate solution (free from any undissolved particles, *i.e.*, saturated), 5 ml of 4 per cent. w/v potassium iodide solution and 10 drops of 1 per cent. starch indicator solution were added. The mixture was titrated with 0·1 per cent. w/v N-bromosuccinimide solution, added dropwise from a microburette, with continuous shaking after each addition, until a blue colour just appeared, and the volume added was noted. A similar series of experiments was carried out with a sodium arsenite solution containing twice the number of molecules of solute present in the first solution. It was found that the reaction was stoicheiometric in presence of sodium hydrogen carbonate at room temperature; the results were—

Volume of sodium arsenite solution (1 millimole per 100 ml) used, ml	10	8	5	2	—	—	—	—	—
Volume of sodium arsenite solution (2 millimoles per 100 ml) used, ml	—	—	—	—	5	4	3	2	1
Titre of N-bromosuccinimide solution (1 millimole per 100 ml), ml	10·1	8	5·1	2	10	8	6	4	2

The equimolecular solutions of sodium arsenite and N-bromosuccinimide (freshly prepared) were then diluted ten-fold with distilled water in 100-ml calibrated flasks. The diluted solutions were titrated as before and gave concordant results.

Method

Reagents—

Potassium iodide solution, 4 per cent. w/v, aqueous.

Sodium hydrogen carbonate solution, 10 per cent. w/v, aqueous—Filter to remove any undissolved solute, *i.e.*, use a saturated solution.

N-Bromosuccinimide solution, 0·1 per cent. w/v, aqueous—Freshly prepare this solution before use.

Starch indicator solution, 1 per cent.

Procedure—

To an accurately measured volume, *e.g.*, 5 ml, of the sodium arsenite solution in a 50-ml conical flask add 5 ml of 10 per cent. sodium hydrogen carbonate solution, *i.e.*, an equal volume, 5 ml of 4 per cent. potassium iodide solution and 10 drops of starch indicator solution. Titrate the mixture with N-bromosuccinimide solution, added dropwise from a microburette graduated to 0·01 ml, with continuous shaking. The end-point is reached when the last drop of titrant added produces a permanent blue colour. Calculate the sodium arsenite content of the sample solution from the expression—

$$\text{Sodium arsenite present, mg or } \mu g = 129{\cdot}92\ vc/178,$$

in which v is the titre of N-bromosuccinimide solution in millilitres and c is the concentration of the N-bromosuccinimide solution in milligrams or micrograms per millilitre.

Applications of the Method

Determination of sodium arsenite—

A 0·5 per cent. stock solution of sodium arsenite was prepared by dissolving 0·5 g of pure sodium arsenite, $NaAsO_2$, obtained from E. Merck and Co., Darmstadt, Germany, in hot distilled water; the solution was allowed to cool and was then diluted with distilled water to 100 ml in a calibrated flask. Solutions containing 0·4, 0·3, 0·2 and 0·1 per cent. of sodium arsenite were prepared by suitable dilution of portions of this solution. The sodium arsenite contents of these pure solutions were then determined by the proposed method; the results, each of which is the mean of at least two determinations, are shown in Table I.

Table I

Recovery of sodium arsenite by the proposed method

Concentration of sodium arsenite solution, % w/v	Volume of solution used, ml	Sodium arsenite content, mg	Titre of 0·1 per cent. w/v N-bromosuccinimide solution, mg	Sodium arsenite found, mg	Error, %
0·5	1	5	6·86	5·01	+0·20
	2	10	13·78	10·06	+0·60
0·4	1	4	5·47	3·99	−0·25
	2	8	10·99	8·02	+0·25
0·3	1	3	4·12	3·01	+0·33
	2	6	8·23	6·01	+0·17
	3	9	12·36	9·02	+0·22
0·2	1	2	2·76	2·01	+0·50
	2	4	5·50	4·01	+0·25
	3	6	8·23	6·01	+0·17
	4	8	10·99	8·02	+0·25
	5	10	13·72	10·01	+0·10
0·1	1	1	1·37	1·00	±0·00
	2	2	2·74	2·00	±0·00
	3	3	4·11	3·00	±0·00
	4	4	5·47	3·99	−0·25
	5	5	6·86	5·01	+0·20
	6	6	8·22	5·98	−0·33
	7	7	9·60	7·01	+0·14
	8	8	10·99	8·02	+0·25
	9	9	12·35	9·01	+0·11
	10	10	13·71	10·01	+0·10

A series of experiments was carried out in which the 0·5 per cent. sodium arsenite solution was analysed by the proposed method, 0·1 *N* N-bromosuccinimide being used, and by titration against 0·1 *N* iodine. The results, each of which is the mean of at least two determinations, are shown in Table II.

Table II

Comparison of results for sodium arsenite by the proposed method and by titration against iodine solution

1 ml of 0·1 *N* N-bromosuccinimide or iodine ≡ 6·496 mg of sodium arsenite

Volume of 0·5 per cent. w/v sodium arsenite solution used, ml	Sodium arsenite content, mg	With 0·1 *N* N-bromosuccinimide as titrant: Titre, ml	Sodium arsenite found, mg	Error, %	With 0·1 *N* iodine as titrant: Titre, ml	Sodium arsenite found, mg	Error, %
10	50	7·80	50·67	+1·34	7·60	49·37	−1·26
5	25	3·90	25·33	+1·32	3·80	24·68	−1·28
4	20	3·10	20·14	+0·70	3·00	19·49	−2·25
3	15	2·29	14·88	−0·80	2·40	15·59	+3·93
2	10	1·55	10·07	+0·70	1·49	9·68	−3·20
1	5	0·77	5·00	±0·00	0·80	5·20	+4·00

Table III

Recovery of arsenite in presence of arsenate

Volume of 0·05 per cent. w/v sodium arsenite solution used, ml	Volume of sodium arsenate solution used, ml	Sodium arsenite content, mg	Sodium arsenate content, mg	Titre of 0·1 per cent. w/v N-bromo-succinimide solution, ml	Sodium arsenite found, mg	Error, %
Arsenite solution mixed with 5 per cent. arsenate solution—						
10	10	5	500	6·95	5·07	+1·40
8	4	4	200	5·57	4·07	+1·75
6	2	3	100	4·18	3·05	+1·67
Arsenite solution mixed with 0·05 per cent. arsenate solution—						
4	8	2	4	2·78	2·03	+1·50
4	4	2	2	2·78	2·03	+1·50
Arsenite solution mixed with 0·005 per cent. arsenate solution—						
10	10	5	0·5	6·96	5·07	+1·40
4	4	2	0·2	2·78	2·03	+1·50
2	2	1	0·1	1·40	1·02	+2·00

Table IV

Recovery of arsenious oxide by the proposed method

Arsenious oxide content of sample, mg or $\mu g = 197{\cdot}84\ vc/356$, where v and c are as defined on p. 291

Volume of arsenious oxide solution used, ml	For 0·1 per cent. w/v arsenious oxide solution: Arsenious oxide content, mg	Titre of 0·1 per cent. w/v N-bromo-succinimide solution, ml	Arsenious oxide found, mg	Error, %	For 0·01 per cent. w/v arsenious oxide solution: Arsenious oxide content, μg	Titre of 0·1 per cent. w/v N-bromo-succinimide solution, ml	Arsenious oxide found, μg	Error, %
5	5	9·04	5·02	+0·40	500	0·90	500·16	+0·03
4	4	7·23	4·02	+0·50	400	0·72	400·13	
3	3	5·42	3·01	+0·33	300	0·54	300·09	
2	2	3·62	2·01	+0·50	200	0·36	200·06	
1	1	1·81	1·01	+1·00	100	0·18	100·03	

The experimental error, as deduced from the results in Tables I and II, does not exceed ±2 per cent.

DETERMINATION OF ARSENITE IN PRESENCE OF ARSENATE—

A standard arsenite solution was prepared by diluting 10 ml of 0·5 per cent. sodium arsenite solution with distilled water to 100 ml in a calibrated flask. Similarly, standard arsenate solution was prepared by dissolving 5 g of sodium arsenate in 100 ml of water, and portions of this solution were diluted 100-fold and 1000-fold. Measured volumes of these solutions were mixed, and the arsenite in the mixtures was determined by the proposed method; the results are shown in Table III.

DETERMINATION OF ARSENIOUS OXIDE—

Before the proposed method was applied to the determination of arsenious oxide, we decided to verify the reaction between N-bromosuccinimide and arsenious oxide from a quantitative point of view. It was assumed that one molecule of arsenious oxide was oxidised by two molecules of N-bromosuccinimide. Accordingly, a solution containing 0·19784 g (1 millimole) of arsenious oxide per 100 ml was titrated against solutions containing 0·178 and 0·356 g (1 and 2 millimoles, respectively) of N-bromosuccinimide. It was found that the reaction between arsenious oxide and N-bromosuccinimide was quantitative in the molecular concentrations expected; the results were—

Volume of arsenious oxide solution (1 millimole per 100 ml) used, ml	10	5	4	3	2	1
Titre of N-bromosuccinimide solution (1 millimole per 100 ml), ml	19·95	9·96	7·99	6	4	2
Titre of N-bromosuccinimide solution (2 millimoles per 100 ml), ml	10	5·05	4·02	3·03	2·03	1·01

Liquor arsenicalis containing 1 per cent. of arsenious oxide was prepared as described in the British Pharmacopoeia, 1932. It was diluted 10-fold and 100-fold with distilled water in 100-ml calibrated flasks to give solutions containing 1 mg and 100 μg of arsenious oxide per ml. These solutions were analysed by the proposed method; the results are shown in Table IV.

A series of comparative analyses was carried out on the Liquor arsenicalis by the proposed method and by the iodine method[16]; the results are shown in Table V.

TABLE V

COMPARISON OF RESULTS FOR ARSENIOUS OXIDE BY THE PROPOSED METHOD AND BY TITRATION AGAINST IODINE SOLUTION

1 ml of 0·1 *N* N-bromosuccinimide or iodine ≡ 4·946 mg of arsenious oxide

Volume of 1 per cent. Liquor arsenicalis used, ml	Arsenious oxide content, mg	With 0·1 *N* N-bromosuccinimide as titrant: Titre, ml	Arsenious oxide found, mg	Error, %	With 0·1 *N* iodine as titrant: Titre, ml	Arsenious oxide found, mg	Error, %
10	100	20·60	101·89	+1·89	20·00	98·92	−1·08
5	50	9·99	49·41	−1·18	10·30	50·94	+1·88
4	40	8·08	39·96	−0·10	8·20	40·56	+1·40
3	30	6·06	29·97	−0·10	6·20	30·67	+2·23
2	20	4·04	19·98	−0·10	4·10	20·28	+1·40
1	10	2·02	9·99	−0·10	2·10	10·39	+3·90

DISCUSSION OF RESULTS

The accuracy of the iodine method depends mainly on the concentration of arsenious oxide in the sample solution. The results in Tables II and V show that the iodine method is only accurate to within ±4 per cent., especially when the amount of arsenite in the sample is as small as 5 mg. The experimental error was somewhat higher than expected because the standard iodine solution exerted an appreciable vapour pressure of iodine[17] during titration and therefore decreased slightly in concentration; iodine was also lost by volatilisation, particularly in hot weather.

The results in Tables I and IV show that the proposed method surpasses the generally accepted iodine method in accuracy and sensitivity. Titrations with 0·1 per cent. N-bromosuccinimide solution are preferable to those with the less stable standard iodine solution, which requires standardisation before use.

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Notes

A SENSITIVE METHOD FOR DETECTING SUGARS ON PAPER CHROMATOGRAMS

Ek and Hultman[1] developed a colorimetric method for determining glucose in body fluids; they used the condensation reaction between the sugar and *p*-aminobenzoic acid or *m*-aminophenol in an almost water-free solution of glacial acetic acid to produce a coloured product. On the basis of their findings, an attempt was made in this laboratory to use a solution of *p*-aminobenzoic acid in glacial acetic acid for the identification of sugars separated by paper chromatography. The sugars used were raffinose, sucrose, lactose, glucose, galactose, fructose, mannose, xylose and rhamnose.

Development of the method

A 0·002-ml portion of a 0·2 per cent. solution, *i.e.*, 4 μg, of each sugar was placed on a piece of Whatman filter-paper, 10 inches × 10 inches, especially prepared for chromatographic work, the spots being about 3·5 cm from one of the edges of the paper and about 2·0 cm apart. A mixture of ethyl acetate, acetic acid and water (3:1:3) was used as developing solvent.[2] About 3 hours were needed for the solvent front to travel to the opposite edge of the paper when subjected to a descending-solvent technique. After completion of the run, the paper was dried for about 15 minutes in a current of air at room temperature; about 30° C. The dried paper was sprayed with a 1·0 per cent. solution of *p*-aminobenzoic acid in glacial acetic acid and heated at 105° C for about 10 minutes. Under these conditions, rather faint brownish spots for glucose, galactose and mannose and a distinct pinkish brown spot for xylose appeared on an extremely pale brownish yellow background; no spots were formed by the other sugars.

An attempt was then made to utilise the general principles of the chromatographic identification of sugars by converting the sugars to furfural or its derivatives and forming coloured products with *p*-aminobenzoic acid. As oxalic acid can be used to convert hexoses to ω-hydroxymethylfurfural[3], the use of this acid was tried. The spraying reagent could not be prepared by dissolving oxalic acid in a solution of *p*-aminobenzoic acid in *n*-butyl or *iso*butyl alcohol or any other common organic solvent, as an insoluble derivative appeared when the oxalic acid (0·5 g) was added to a 1 per cent. solution of *p*-aminobenzoic acid. A double-spraying technique was therefore used. After the chromatogram had been run and dried as before, it was uniformly sprayed with a 1 per cent. solution of *p*-aminobenzoic acid in *iso*butyl alcohol and dried at room temperature for about 15 minutes in a current of air; it was then sprayed with a 0·5 per cent. solution of oxalic acid in *iso*butyl alcohol and immediately heated at 105° C for about 10 minutes. In presence of 4-μg amounts of sugars, distinct chocolate-brown to yellowish brown spots on an

extremely pale greyish brown background were formed by glucose, galactose, mannose and xylose. Spots formed by sucrose, lactose and rhamnose were faint, and those formed by raffinose and fructose were extremely faint. In presence of 2-μg amounts of sugars, only glucose, galactose, mannose and xylose could be detected; the other sugars formed no spots.

However, when a 0·5 per cent. solution of oxalic acid in glacial acetic acid was used for the second spraying, 2-μg amounts of all the sugars formed easily detectable spots, although that formed by raffinose was somewhat faint; each spot had a sharp boundary. When an aniline - phthalic acid reagent[4] was used as spraying reagent and the amount of each sugar present was 4 μg, reasonably distinct spots were formed only by glucose, galactose, mannose and xylose, spots for the other sugars being either absent or extremely faint. Under identical conditions, spots developed with the proposed reagent were more distinct. When the amount of each sugar present was 1 μg, spots formed by glucose, galactose, mannose, xylose and rhamnose could be detected with the proposed reagent, whereas with the aniline - phthalic acid reagent, the only discernible spot was that formed by xylose.

A mixture of 2-μg amounts of raffinose, glucose, xylose and rhamnose, which have appreciably different R_F values in ethyl acetate - acetic acid - water mixture, was subjected to paper chromatography. Each of the separated sugars could be detected with the proposed spraying reagent; the spot formed by raffinose was extremely faint.

Conclusions

With 1-μg amounts of the sugars tested, the spots formed by glucose, mannose, xylose and rhamnose were faint but detectable. With 2-μg amounts, the intensity of the spots improved and with 4-μg amounts all spots were distinct. When the amount of each sugar present was increased to 8 μg (the largest amount tested), the spots were even more distinct.

The spots obtained by the proposed method are on an extremely pale greyish brown background and persist for several days without any appreciable reduction in colour intensity. The method is most sensitive and can be applied to a wide variety of sugars; it is hoped to apply it to the detection of various sugars in biological fluids, especially in human sweat.

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THE DETERMINATION OF GALLATES IN EDIBLE FATS

Under the Antioxidants in Food Regulations, 1958, limited amounts of *n*-propyl, *n*-octyl and *n*-dodecyl gallates are permitted in certain foods. To avoid the expenditure of unnecessary effort on samples eventually proving to be perfectly satisfactory, a rapid and reasonably accurate sorting test, with a recovery of at least 95 per cent., was desirable. It was considered sufficient to determine total gallate quantitatively.

Experimental

The normal colorimetric method for determining gallate, involving the use of iron, was unsatisfactory in methanolic solution. However, it was found that when a gallate in 95 per cent. methanol was shaken with solid ammonium ferrous sulphate, a clear and stable blue solution was obtained; such solutions obeyed Beer's law over the range 0·0 to 0·8 mg of gallate, maximum absorption in the visible region occurring at 5800 A. The absorption spectrum for *n*-propyl gallate is shown in Fig. 1; the shape of the curve is characteristic of all three gallates, but the optical density at any given concentration depends on the particular ester present.

Application of this reaction to extracts of fats in 95 per cent. methanol produced abnormal results, owing to the development of turbidity in the solutions and to the synergistic effects of other extracted matter. It was found that these effects could be nullified by shaking the extract with analytical-reagent grade calcium carbonate, which neutralised any extracted acidity and assisted in the clarification of the solution by coagulating some of the extraneous matter. The addition of 10 per cent. of analytical-reagent grade acetone after filtration further stabilised the solution, and recovery experiments showed that there was no detectable loss of gallate during treatment with calcium carbonate and that the colour was not affected (except by dilution) by addition of acetone. The use of solid ammonium ferrous sulphate removed those difficulties attendant on the use of an unstable reagent, such as ferrous tartrate solution.

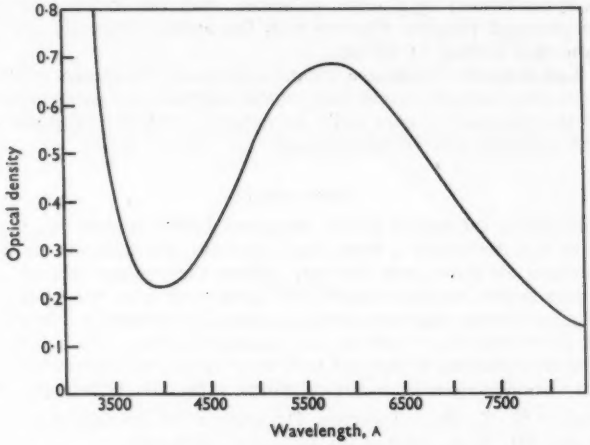

Fig. 1. Absorption spectrum of solution containing ammonium ferrous sulphate and 0·44 mg of *n*-propyl gallate in 95 per cent. methanol; final volume 11 ml

In a method described by Vos, Wessels and Six,[1] large volumes of fat were extracted with relatively small amounts of solvent; this necessitated several manipulations in order to achieve reasonable recovery. We have found that oils can be extracted directly with 95 per cent. methanol at 40° to 45° C and that solid fats can be extracted similarly when diluted with an equal volume of liquid paraffin. Under these conditions the recovery of added gallates in two extractions was 95 per cent.

Method

Apparatus—

Extraction vessels—The extraction vessels used consist of boiling tubes, each of which has a bulb of capacity 10 to 12 ml blown in its side at the bottom (see Fig. 2); they permit the separation of an upper layer simply by tilting the tube.

Fig. 2. Extraction vessel

Reagents—

Unless otherwise stated, all materials must be of recognised analytical grade.

Liquid paraffin—B.P. grade.
Methanol, 95 per cent.
Ammonium ferrous sulphate.
Calcium carbonate.
Acetone—Shake with calcium carbonate for 1 minute, and filter.

PROCEDURE—

Vigorously shake 10 g of warm liquid sample with 25 ml of 95 per cent. methanol for 1 minute in a vessel of the type shown in Fig. 2. (For solid samples, use a 5-g portion *plus* 5 ml of liquid paraffin.) Place in a water bath at 40° to 45° C, and allow to separate for about 15 minutes (separation into clear layers is unlikely and unnecessary). Pour the upper layer into a 50-ml calibrated flask, repeat the extraction with 20 ml of 95 per cent. methanol, again transfer the upper layer to the flask, and dilute to the mark. Add 1 g of calcium carbonate to the contents of the flask, shake for 30 seconds, filter through a Whatman No. 1 filter-paper, and reject the first few millilitres of filtrate. (The amount of calcium carbonate added is not critical, but must be sufficient to ensure a clear filtrate at this stage.) To 10 ml of filtrate add exactly 1 ml of acetone and about 10 mg of powdered ammonium ferrous sulphate, and shake for 1 minute. Set aside for 30 minutes to attain full colour development, and measure the optical density at 5800 A in 1-cm cells with a Unicam SP500 spectrophotometer. For extremely dilute solutions larger cells may be necessary.

Calculate the amount of gallate present per 11 ml of final solution from the expression—

$$\text{Gallate present, mg} = dk,$$

in which d is the optical density measured in 1-cm cells and k has the value 0·622, 0·785 or 0·952 for *n*-propyl, *n*-octyl or *n*-dodecyl gallate, respectively. (It is recommended that these factors be determined for each batch of reagents.)

RESULTS

The recoveries of gallates from various oils and fats by the proposed method are shown in Table I. When necessary, the esters can be distinguished from each other by a method such as that described by Vos, Wessels and Six.[1]

TABLE I

RECOVERY OF ADDED GALLATE FROM OILS AND FATS

Sample	Gallate added, p.p.m.	Recovery of—: *n*-propyl gallate, %	*n*-octyl gallate, %	*n*-dodecyl gallate, %
Olive oil	100	95·7	96·2	95·4
	200	95·2	95·7	95·7
Lard	100	96·0	96·5	95·3
	200	96·2	96·0	95·8
Dripping	100	95·6	96·8	96·5
	200	96·1	96·3	95·7
Olive oil *plus* dripping ..	100	96·7	94·7	95·5
	200	95·9	95·5	96·1

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SPECIFIC MASKING BY ACETYLACETONE IN TITRATIONS WITH ETHYLENEDIAMINETETRA-ACETIC ACID

THE use of acetylacetone as a masking agent to improve the selectivity in potentiometric titrations with ethylenediaminetetra-acetic acid (EDTA) was first described by Fritz, Richard and Karraker.[1] During investigations into the application of certain masking agents in EDTA titrations with xylenol orange as indicator,[2] it was found that acetylacetone (2:4-pentanedione) could be used as a masking agent for aluminium, iron^{III}, beryllium, palladium and uranium when determining

zinc or lead. In Table I are summarised the masking effects of acetylacetone[1,3] on certain cations under the conditions used for titrating zinc or lead with xylenol orange as indicator.

TABLE I

MASKING EFFECT OF ACETYLACETONE ON CERTAIN CATIONS

Cations masked—

Fe^{3+}, Al^{3+}, Be^{2+}, Pd^{2+}, UO_2^{2+}

Cations partly masked—

Cu^{2+}, Hg^{2+}, Cr^{3+}, Ti^{4+}

Cations not masked—

Zn^{2+}, Pb^{2+}, Mn^{2+}, Ni^{2+}, Co^{2+}, Th^{4+}, Cd^{2+}, La^{3+}, Sn^{2+}, Bi^{3+}, Ce^{3+}

The complexes formed by acetylacetone with aluminium, iron, beryllium, palladium and uranium are much more stable than either the metal - xylenol orange complex or the metal - EDTA complex. Lead and zinc do not form stable complexes with acetylacetone and can therefore be titrated in the presence of any of the metals just mentioned without interference, provided that acetylacetone is added to the solution before titration. Zinc forms a weak complex with acetylacetone, especially in warm solution, but the zinc - EDTA complex is so strong that zinc can be removed from its complex with acetylacetone by titration with EDTA. Iron and uranium both produce somewhat intense colours with acetylacetone, and this makes normal titration and end-point detection impossible. The addition of an organic solvent, such as nitrobenzene or chlorobenzene, permits the colour to be extracted into the organic layer, and the end-point can then be detected in the aqueous layer.

Molybdate can also be masked with acetylacetone, but the complex so formed is stable only in strongly acid solution, and the reaction is of practical use only when titrating a metal such as bismuth.[2,4,5] In absence of acetylacetone, molybdate forms a weak complex with xylenol orange and also a precipitate with bismuth at the optimum pH for determining bismuth by titration against EDTA with xylenol orange as indicator.

TABLE II

EFFECTS OF VARIOUS SALTS ON TITRATION OF LEAD AND ZINC NITRATES WITH 0·1 *M* EDTA

Sample	Titre of 0·1 *M* EDTA for— solution containing zinc nitrate, ml	solution containing lead nitrate, ml
Solution A*	49·65	49·5
Solution B†	49·65	49·5
Solution B *plus* 1 g of $Fe(NO_3)_3.9H_2O$	49·7	49·55
Solution B *plus* 1 g of $Al(NO_3)_3.9H_2O$	49·65	49·5
Solution B *plus* 1 g of $UO_2(NO_3)_2.6H_2O$		
Solution B *plus* 1 g of $BeCO_3$ dissolved in nitric acid		
Solution B *plus* 1 g of $Pd(NO_3)_2$		

* Fifty millilitres of a solution containing zinc or lead, as nitrate.
† Solution A *plus* 5 ml of acetylacetone.

METHOD

PROCEDURE FOR DETERMINING ZINC OR LEAD—

In absence of other metals[2]—To 50 ml of a neutral or slightly acid solution of the sample add 2 ml of 5 *N* nitric acid and 5 g of hexamine or sufficient to produce a pH of 5 to 6. Titrate against 0·1 *M* EDTA with 5 drops of a 0·2 per cent. aqueous solution of xylenol orange as indicator until the colour changes from wine-red to pure yellow.

In presence of beryllium, palladium or aluminium—Proceed as described above, but add 5 ml of acetylacetone before the solution is buffered with hexamine. Acetylacetone forms an almost colourless insoluble precipitate with any of these metals.

In presence of iron or uranium—To 50 ml of a neutral or slightly acid solution of the sample in a stoppered 500-ml flask add 2 ml of 5 *N* nitric acid and a few drops of 20-volume hydrogen peroxide to oxidise any ferrous salt present. (In absence of iron this can be omitted.) To the

clear and cold solution add 5 ml of acetylacetone and then 5 g of hexamine to adjust the pH to between 5 and 6. Add 50 ml of nitrobenzene, and shake vigorously to extract the metal - acetylacetone complex into the organic layer. Dilute to 300 ml with water, add 1 ml of xylenol orange indicator solution, and titrate immediately with 0·1 *M* EDTA. Add titrant slowly as the end-point is approached, and observe the colour change in the aqueous layer.

The organic layer should not be removed, as it contains some zinc.

PROCEDURE FOR DETERMINING BISMUTH IN PRESENCE OF MOLYBDATE—

To the clear strongly acid solution containing bismuth and molybdate add 5 ml of acetylacetone. Mix well, and set aside until the acetylacetone - molybdate compound has been completely precipitated. Adjust the pH of the solution to between 1 and 1·5 with 5 *N* sodium hydroxide or 5 *N* ammonium hydroxide, add 1 ml of xylenol orange indicator solution, and titrate with 0·1 *M* EDTA. Towards the end of the titration, the colour of the solution begins to fade. At this point, add sufficient alkali dropwise to restore the red - orange colour of the bismuth - xylenol orange complex, and then complete the titration.

RESULTS

Table II shows the results found when solutions containing zinc nitrate and lead nitrate were titrated with 0·1 *M* EDTA in the presence of acetylacetone and various metallic salts.

We thank the Directors of Hopkin and Williams Ltd. for permission to publish this Note.

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DETERMINATION OF SULPHIDE SULPHUR IN MINERALS

A RAPID and accurate method for determining sulphide sulphur in minerals containing the sulphides of lead, zinc, bismuth, manganese, nickel, cobalt, silver, etc., has been described.[1] This method consisted in reducing the mineral with hydriodic acid to evolve hydrogen sulphide, which was swept off in a current of hydrogen or nitrogen and absorbed by a suspension of cadmium hydroxide, the sulphide being determined iodimetrically. It was pointed out, however, that this method could not be applied to the analysis of pyrite and chalcopyrite, as the reaction is extremely slow.

We have found that the reaction can be hastened to completion by using a more concentrated solution of hydriodic acid (analytical-reagent grade, sp.gr. 1·7, containing from 54 to 56 per cent. of hydrogen iodide). Solution of such minerals as pyrite or chalcopyrite is smooth and complete, provided that a pellet of mercury is added to the sample. It is necessary to heat the contents of the reaction vessel gently. The free iodine usually present in the hydriodic acid can easily be reduced by adding a few crystals of sodium hypophosphite; this gives a colourless reagent solution.

METHOD

PROCEDURE—

Place 50 to 100 mg of powdered mineral (it need not necessarily be finely pulverised) in the reaction vessel,[1] add a small pellet of mercury, and displace the air in the apparatus by hydrogen. Add about 5 ml of hydriodic acid, and gently warm the flask. Sweep off the hydrogen sulphide, absorb it in an alkaline suspension of cadmium hydroxide, and carry out the determination as described previously.[1] The entire procedure can be carried out in 1 hour.

After removal of hydrogen iodide by boiling with concentrated sulphuric acid, the residue in the reaction vessel can be used for determining other components of the sample by standard methods.

RESULTS

Some representative samples of sulphide minerals were analysed by the proposed method; the results are shown in Table I.

TABLE I

SULPHIDE SULPHUR FOUND IN VARIOUS MINERALS

Mineral	Sulphur content of pure mineral, %	Sulphur found, %
Iron pyrites	53·42	52·16
Chalcopyrite	34·89	33·83
Stibnite	28·32	28·30
Realgar	29·93	29·11
Orpiment	39·05	38·36
Sphalerite	32·86	31·65

It can be seen that the sulphide sulphur present can be determined with reasonable accuracy. The results of duplicate determinations agreed to within 0·5 per cent., and determinations of total sulphur by wet oxidation gave the same values as those found by the proposed method, provided that the mineral had not undergone any appreciable oxidation.

DISCUSSION OF THE METHOD

In view of the commercial and practical importance of determining sulphur in such minerals as pyrite, the proposed method provides a valuable and rapid procedure. There is no possibility of any interference by other radicles, such as iron, and no danger of contamination by the products of combustion when gas flames are used (these combustion products usually contain oxides of sulphur, which are reported to be taken up avidly by alkali fluxes and oxidising solutions).[2]

The preparation of a solution in determining the sulphur content of antimony sulphide minerals is said to be difficult,[3] but a naturally occurring sample of stibnite can be analysed for sulphur in 1 hour by the proposed method; the result obtained agrees with the calculated value. This is also true for arsenic sulphide minerals.

If pyrite is treated with concentrated hydriodic acid alone, about 14 per cent. of the sulphur present is evolved as hydrogen sulphide, whereas, in presence of mercury, the entire amount is recovered as hydrogen sulphide. In presence of mercury, even a more dilute reagent solution, such as that prepared by mixing concentrated hydrochloric acid and potassium iodide solution,[1] will react with pyrite to evolve hydrogen sulphide quantitatively. By virtue of its reducing action and ability to form complexes with metallic derivatives and so hold them in solution, hydrogen iodide can react with such a mineral as pyrite, which is insoluble in a non-oxidising acid, *e.g.*, concentrated hydrochloric acid.[4]

Solution of pyrite in presence of mercury is due to galvanic effects rather than to chemical reaction. The reaction is slow when a mercury salt (mercuric chloride) is used instead of metallic mercury. Any metal more electropositive than iron, *e.g.*, lead, tin, copper or silver in powdered form, will have the same effect as mercury, whereas zinc and aluminium, which are more electronegative than iron, will not. All these metals specified dissolve rapidly in the acid, but, mercury is more electropositive than most of them and is easy to handle; its use is therefore preferred. Further work is in progress to ascertain the role of mercury and other metals in the solution of pyrite by hydriodic acid.

We thank Professor M. R. A. Rao for his valuable suggestions and keen interest in this work.

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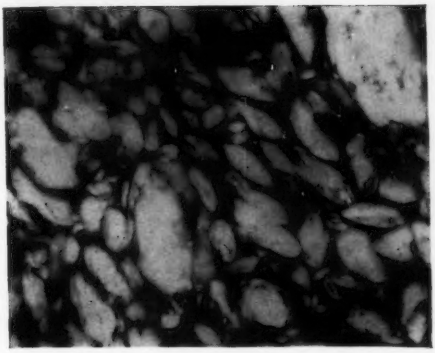
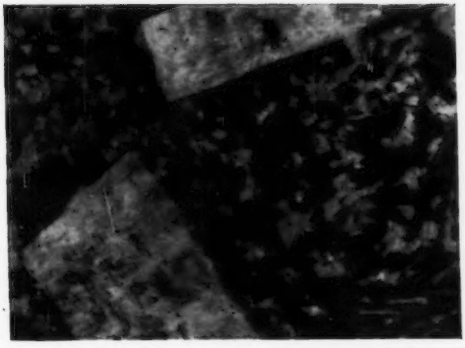
THE STAINING OF CAST HIGH EXPLOSIVES FOR OBSERVATION OF THE CRYSTALLINE STRUCTURE

THE nature of the microstructure of cast high explosives is of importance, since in some instances it can be correlated with the explosive properties.[1,2,3] Various observational techniques have been described.



(*a*)

(*b*)



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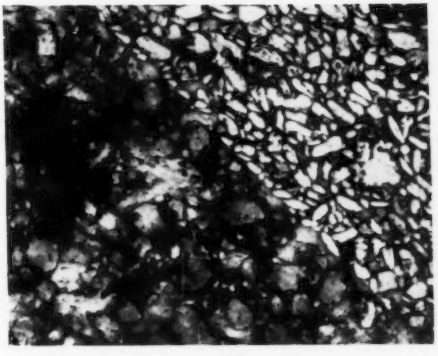

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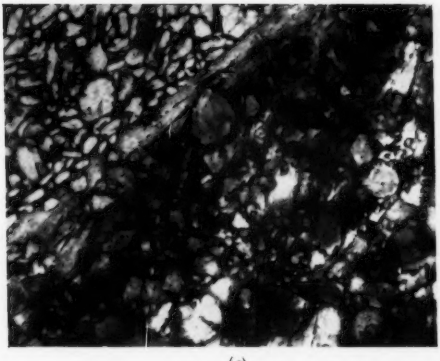

(*e*)

Fig. 1. Typical photomicrographs: (*a*) RDX in matrix of TNT; (*b*) PETN in matrix of TNT; (*c*) 60/40 tetryl - TNT; (*d*) and (*e*) boundary between 60/40 RDX - TNT and 50/50 amatol

[To face p. 300

Williamson[2,4] has used two methods, a microtome technique in which a thin film of explosive adhering to a backing material is examined under a polarising microscope and a "film and cast" technique in which a cast of the surface features is made and studied. Another approach is to use a stain to show up one component. Pristera[5] has investigated the reactions between trinitrotoluene (TNT) and the hydroxides of sodium, potassium and ammonium, which produce a reddish colour.[6,7] He found that *N* potassium hydroxide in diethylene glycol can be successfully used as a staining agent on an explosive specimen cooled by solid carbon dioxide.

A more convenient staining technique can be based on the coloured complexes formed between some nitrobodies and organic bases.[8] The most convenient base for this purpose has been found to be dimethylaniline, which gives a red colour with TNT, orange with tetryl and picric acid and no colour with the other common high explosives. The method used is to cut a section about 3 mm thick from the end of a casting by means of a fine saw and to smooth one face of this by rubbing gently on glass-paper. The specimen is then glued by this face to a microscope slide and its thickness reduced to about 1 mm with a microtome. The surface is lightly wiped first with a cotton-wool pad moistened with acetone and then one wetted with dimethylaniline. The specimen is examined under a microscope by transmitted white light.

A selection of typical photomicrographs obtained by this method is shown in Fig. 1. Figs. 1 (*a*) and 1 (*b*) show *symo*-trimethylene trinitramine (RDX) and pentaerythritol tetranitrate (PETN), respectively, in a matrix of TNT. Fig. 1 (*c*) shows the structure of 60/40 tetryl - TNT, in which the crystals of tetryl are embedded in a matrix of the eutectic conglomerate.

In investigations of the transition of detonation from one explosive to another good contact between the two compositions is essential, and this can best be done by casting one material on to the other.[9] Figs. 1 (*d*) and 1 (*e*) show about 5 mm of the boundary between 60/40 RDX - TNT and 50/50 amatol. Fig. 1 (*d*) shows a perfect join, but Fig. 1 (*e*) shows what happens when there is a wide temperature difference between the already solidified substance and the second melt. A surface layer rich in TNT is formed,[3] and this can be seen along the interface.

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Book Reviews

Principles of Electrolysis. By C. W. Davies, D.Sc., F.R.I.C. Pp. vi + 30. London: The Royal Institute of Chemistry. 1959. Price 3s. 6d.

This is the first of the monographs for teachers issued by the Royal Institute of Chemistry. The aim of the Institute is to provide "concise and authoritative accounts of selected well-defined topics in chemistry for the guidance of those who teach the subject at G.C.E. Advanced Level and above." It was believed that these monographs would be of value also to a wider readership.

The subject of this first booklet is of undoubted interest to analysts, for electrolytic processes often provide us with an elegant analytical technique. There are chapters on First Principles, Electrolytic Conduction, The Electrode Reaction and Examples of Electrolytic Processes. There is a short summary of principles, and, in an appendix, a list of standard electrode potentials is given.

Professor Davies and the Royal Institute of Chemistry are to be congratulated on the preparation and publication of this useful little booklet

R. C. Chirnside

CHROMATOGRAPHIC REVIEWS: PROGRESS IN CHROMATOGRAPHY, ELECTROPHORESIS AND RELATED METHODS. VOLUME I. Edited by MICHAEL LEDERER. Pp. x + 276. Amsterdam, New York and Princeton: Elsevier Publishing Co.; London: D. Van Nostrand Co. Ltd. 1959. Price 45s.

Too many new journals and reviews are now being produced to benefit publishers' profits, one fears, rather than the world of science. Yet when they appear, they have to be bought and read for fear of missing something important—which could just as appropriately have appeared in an established journal. It is especially questionable whether or not new publications dealing with techniques rather than subjects are needed.

Chromatographic Reviews, Volume I, was accordingly read against this background of prejudice. However, the high quality of the papers included force one, albeit grudgingly, to admit this volume as an exception. It deserves a place in the library of any institution where chromatography or electrophoresis is practised at all extensively.

This Review is in any event different from most others. The papers were not solicited specifically for the volume, but are reviews that have already been published in the *Journal of Chromatography*. The difference is that all but one of the originals were in French or German, but they re-appear as English translations. For English-speaking readers, therefore, this Review is to some degree a more convenient alternative to the *Journal*. Also included is Neher's valuable book "*Chromatographie von Sterinen, Steroiden und verwandten Verbindungen*," very well translated by P. Oesper of Philadelphia. The other papers have also been excellently translated by Miss A. M. G. Macdonald of Birmingham University. She has, it is true, admitted one brave new word, but it is so expressive and concise that it deserves a place in the language. This is "demixion," meaning the partial separation on paper or a chromatostrip of the components of a solvent mixture used for development, such that there are virtually two solvent fronts, which sometimes improves the discrimination between test substances.

Incidentally, it is a pity that nomenclature in this field has not been standardised. In particular, the word "develop" is used to mean running solvent through the chromatogram and also in the different photographic sense of rendering a colourless spot visible by spraying with a reagent: none of the alternatives, to "locate," "reveal," "detect" or "visualise," is quite as expressive.

The first two papers, by E. Demole on chromatostrips and chromatoplates and by H. Michl on high-voltage electrophoresis, provide admirably concise, critical and well documented reviews of these topics. The third, by L. Reio, on paper chromatography of phenols, mould metabolites and related compounds, covers original work by the author. He has elaborated a kind of mass-production technique by which unknowns are run in six solvent systems and sprayed with eleven reagents. The R_F values of 450 reference compounds are quoted, as well as the colours with various reagents, referred to a colour-pencil chart.

The paper by G. B. Marini-Bettolo and G. C. Casinovi on strychnos and curare alkaloids, although well written, is depressing on account of the complexity and inconclusiveness of the results. Neher's exhaustive treatment of the steroids (with 393 references) was mentioned earlier. There follow two useful papers on plant pigments, by Z. Šesták and by J. B. Harborne (the only English contributor), and the last two are in the inorganic field. H. Hettler has provided a concise but full summary of the paper chromatography of inorganic phosphorus compounds. The final chapter by M. Chemla, on isotopes, is also interestingly written, although it is of less practical value, because only in rare instances can isotopes be completely separated by chromatography or electrophoresis.

The whole book is well produced and commendably free from errors; it constitutes an invaluable reference book for those working on the topics covered or related ones.

E. LESTER SMITH

Publications Received

ORGANIC SYNTHESES. An Annual Publication of Satisfactory Methods for the Preparation of Organic Chemicals. Volume 39. Editor-in-Chief: MAX TISHLER. Pp. viii + 114. New York: John Wiley & Sons Inc.; London: Chapman & Hall Ltd. 1959. Price $4.00; 32s.

THE CHEMISTRY OF PLANT GUMS AND MUCILAGES AND SOME RELATED POLYSACCHARIDES. By F. SMITH and R. MONTGOMERY. Pp. x + 627. New York: Reinhold Publishing Corporation; London: Chapman & Hall Ltd. 1959. Price $18.00; 144s.

THE CHEMICAL ANALYSIS OF AIR POLLUTANTS. By MORRIS B. JACOBS, Ph.D. Pp. xviii + 430. New York and London: Interscience Publishers Inc. 1960. Price $13.50; 102s.

AN INTRODUCTION TO PRACTICAL INFRA-RED SPECTROSCOPY. By A. D. CROSS, B.Sc., Ph.D. Pp. viii + 80. London: Butterworths Scientific Publications. 1960. Price 17s. 6d.

SEPARATION AND IDENTIFICATION OF FOOD COLOURS PERMITTED BY THE COLOURING MATTERS IN FOOD REGULATIONS 1957. By The Association of Public Analysts. Pp. vi + 31. London: The Association of Public Analysts. Price 21s.
(*The Association of Public Analysts, Bank Chambers, 16 Southwark Street, London, S.E.*1.)

LE DOSAGE MICROBIOLOGIQUE DES VITAMINES DU GROUPE B. By JEAN ADRIAN. Les Cahiers Techniques du Centre National de Coordination des Études et Recherches sur la Nutrition et l'Alimentation—IV. Pp. viii + 183. Paris: Centre National de la Recherche Scientifique.

A CONCISE ORGANIC CHEMISTRY. By J. B. GUY, M.A., B.Sc., and S. D. WOODS, M.A. Pp. viii + 501. London and Glasgow: Blackie & Son Ltd. 1960. Price 27s. 6d.

LIAISONS ET PROPRIÉTÉS CHIMIQUES: INTRODUCTION À LA THÉORIE DE LA LIAISON CHIMIQUE. By J. A. A. KETELAAR. Translated into French by M. FAYARD. Pp. x + 372. Paris: Dunod. 1960. Price NF 48

CHEMICAL ANALYSIS: AN ADVANCED TEXT AND REFERENCE. By HERBERT A. LAITINEN. Pp. xiv + 611. New York, Toronto and London: McGraw-Hill Book Company Inc. 1960. Price $12.50; 97s.

WORLD REVIEW OF NUTRITION AND DIETETICS. Edited by GEOFFREY H. BOURNE. Volume I. Pp. viii + 272. London: Pitman Medical Publishing Co. Ltd. 1959. Price 60s.

GLASS TECHNOLOGY. Vol. I, No. 1, February, 1960. Edited by R. W. DOUGLAS. Pp. 52 + Abstracts, 1A–16A. Sheffield: Society of Glass Technology. Subscription price 135s. per volume.

PHYSICS AND CHEMISTRY OF GLASSES. Vol. I, No. 1, February, 1960. Edited by R. W. DOUGLAS. Pp. 38 + Abstracts, 1A–16A. Sheffield: Society of Glass Technology. Subscription price 135s. per volume.
Joint subscription to both the above journals £10 per volume.

INSTRUMENTAL METHODS OF CHEMICAL ANALYSIS. By GALEN W. EWING. Second Edition. Pp. viii + 454. New York, Toronto and London: McGraw-Hill Book Company Inc. 1960. Price $8.90; 69s.

ELECTRONICS FOR SPECTROSCOPISTS. Edited by C. G. CANNON, B.Sc., F.Inst.P. Pp. x + 333. London: Hilger & Watts Ltd. 1960. Price 60s.
Schwarz Memorial Volume.

A HISTORY OF GREEK FIRE AND GUNPOWDER. By J. R. PARTINGTON, M.B.E., D.Sc. Pp. xvi + 381. Cambridge: W. Heffer & Sons Ltd. 1960. Price 70s.

GASANALYSE: METHODEN DER ARBEITSPRAXIS. By Dr. FRITZ BAYER and Dr. GEORG WAGNER. Pp. xii + 288. Stuttgart: Ferdinand Enke Verlag. 1960. Price (paper) DM 56.60; (cloth boards) DM 60.

Erratum

MARCH (1960) ISSUE, p. 217, Fig. 1, label on vertical axis. *For* "mg per 100 ml" *read* "mg per 10 ml."

REPORTS OF THE ANALYTICAL METHODS COMMITTEE OBTAINABLE FROM THE SECRETARY

The Reports of the Analytical Methods Committee listed below may be obtained direct from the Secretary, The Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1 (not through Trade Agents), at the price of 1s. 6d. to members of the Society, and 2s. 6d. to non-members. Remittances must accompany orders and be made payable to "Society for Analytical Chemistry."

Sub-Committee on Dirt in Milk. Report. Determination of Dirt in Milk.

Essential Oils Sub-Committee:

Report No. 1. Estimation of Cineole in Essential Oils. (1) Cajuput and Eucalyptus Oils.
Report No. 2. Physical Constants (1).
Report No. 4. Interim Report on the Determination of Acetylisable Constituents in Essential Oils.
Report No. 5. Determination of Phenols in Essential Oils.
Report No. 7. Determination of Solubilities.
Report No. 9. Determination of Carvone and Menthone.
Report No. 12. Determination of Ascaridole.
Report No. 13. Determination of Esters. (Addendum to Report No. 13, Gratis.)
Report No. 14. Solubility Test for Ceylon Citronella Oil. (Gratis.)
Report No. 15. Determination of Linalol in Essential Oils.
Application of Gas - Liquid Chromatography to Essential-oil Analysis: Interim Report on the Determination of Citronellol in Admixture with Geraniol.

Metallic Impurities in Foodstuffs Sub-Committee:

Report No. 4. Determination of Zinc.
Determination of Lead in Foodstuffs: Tentative Method.

Metallic Impurities in Organic Matter Sub-Committee:

The Determination of Lead.
Notes on Perchloric Acid and its Handling in Analytical Work.

Sub-Committee on the Determination of Unsaponifiable Matter in Oils and Fats and of Unsaponified Fat in Soaps:

Report No. 1. Determination of Unsaponifiable Matter in Oils and Fats.
Report No. 2. Determination of Unsaponified Fat in Soap. *Out of print.*
Report No. 3. Determination of Free Alkali in Soaps.
Report No. 4. Determination of Free Alkali and Silica in Silicated Soaps.
Report No. 5. Determination of Rosin in Soaps.
Report No. 6. Determination of Phenols in Soaps.

Poisons Sub-Committee appointed to investigate Methods of Assay for Various Substances appearing in the Poisons Schedules of the Poisons Regulations, 1935:

Report No. 1. Assay of Lobelia (*Lobelia Inflata*).
Report No. 2. Assay of Gelsemium.
Report No. 3. Assay of Aconite.
Report No. 4. Assay of Yohimba.
Report No. 5. Assay of Jaborandi.
Report No. 6. Assay of Ephedra and of Ephedrine in Nasal Sprays.

Fluorine in Foods Sub-Committee:

Report on the Determination of Fluorine in Foods. (Addendum to this Report, Gratis.)

Sub-Committee on Vitamin Estimations:

Report on the Microbiological Assay of Riboflavine and Nicotinic Acid.
The Determination of Carotene in Green-Leaf Material. Part 1. Fresh Grass.
The Determination of Carotene in Green-Leaf Material. Part 2. Green-Leaf Materials other than Grass. (Gratis.)
The Chemical Assay of Aneurine [Thiamine] in Foodstuffs.
The Microbiological Determination of Thiamine.
The Estimation of Vitamin B_{12}.

Vitamin-E Panel:

The Determination of Tocopherols in Oils, Foods and Feeding Stuffs.

Tragacanth Sub-Committee:

Report No. 1. Evaluation of Powdered Tragacanth.
Report No. 2. Evaluation of Flake Tragacanth.

Soapless Detergents Sub-Committee:

Examination of Detergent Preparations.

Meat Extract Sub-Committee:

Analysis of Meat Extract.
Determination of Gelatin in Meat Extract and Meat Stocks: Interim Report.

Pesticides Residues in Foodstuffs Sub-Committee:

Determination of Small Amounts of Total Organic Chlorine in Solvent Extracts of Vegetable Material.